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Abstracts

DEVELOPMENT OF THE FIRST MULTI-TARGET DIRECTED LIGANDS, NEUROTROPHIN MIMETICS, A NEW APPROACH IN THE TREATMENT OF NEURODEGENERATIVE DISEASES

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Numerous studies have been published about the implication of the neurotrophin tyrosine kinase receptor - TrkB in the pathogenesis of several neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, Multiple Sclerosis and motor neuron disease[1,2,3]. Brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5) activate the TrkB receptor with high potency and specificity, promoting neuronal survival, differentiation and synaptic function. On the other side, activation of the p75 neurotrophin receptor (a member of the tumour necrosis factor receptor family) can activate several signalling cascades. The TRAF6 (TNF Receptor Associated Factor 6) cascade which is inducing cell death[4,5], and the RIP2 (receptor-interacting protein 2) cascade which propagates cell survival[5].

Based on all these findings we developed two strategies in order to design and synthesize small molecules, able to prevent neuronal death and to increase neuroregeneration. The first strategy was to use the main structural characteristics of LM22A-4[6], a known activator of the TrkB receptor, modifying the third position of it (by introducing N-alkyl or N-cycloalkyl piperidine substituent) in order to obtain the compounds which will be not only ligands for TrkB receptor but also act as partial 5-HT4 receptor agonists. There are evidences that partial 5-HT4 receptor agonist (RS67333) can increase the concentration of BDNF[7]. The second strategy was to modify LM11A-31[8], a molecule that is able to activate a specific cascade of the p75 neurotrophin receptor, inducing cell survival, and to merge it with TrkB activator, LM22A-4. The reason why we wanted to target these two receptors at the same time, is due to the fact that p75 receptor is upregulated in every neurodegenerative condition and by combining these two activations we believe that we can achieve a synergistic effect, while on the other side, we will prevent proneurotrophins to bind to the p75 receptor and to cause neuronal death[4].

As a result of our study, we have developed two new datasets of small molecules, potential TrkB/5-HT4 receptors ligands and TrkB/p75 receptors ligands, which will be used for further biological research and hit to lead optimisation studies.

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A TOUCHSTONE FOR MACROPHAGE MIGRATION INHIBITORY FACTOR BINDER

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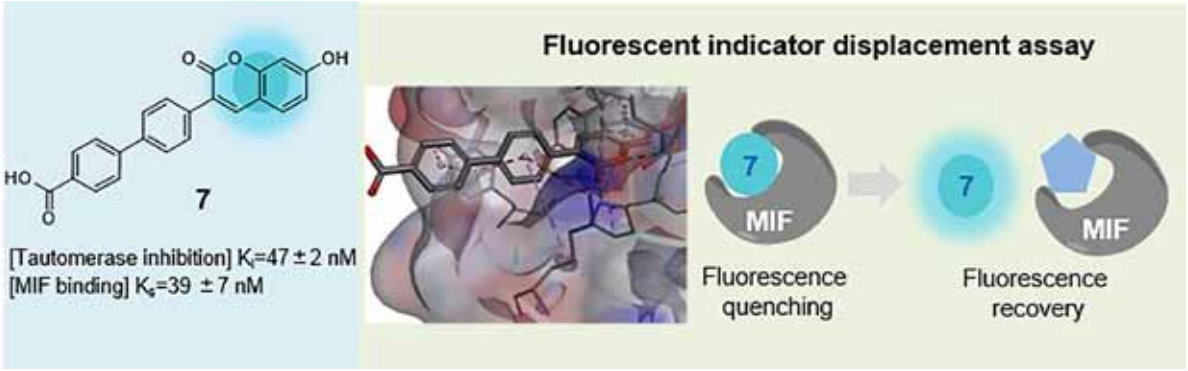
Macrophage migration inhibitory factor (MIF) is a cytokine with key roles in inflammation and cancer. MIF binding to the CD74 receptor activates the MAPK and the PI3K signaling pathways thus stimulating cell survival and proliferation. MIF thus gained interests as a drug target. However, the most widely used assays to identify MIF binding molecules, which rely on MIF tautomerase enzyme activity, were troubled by poor reproducibility, thus creating a need for alternative methods to study MIF binding. In this study, we identified a 7-hydroxycoumarin with high affinity for the MIF tautomerase active site (K_i = 47±2 nM) that binds with concomitant quenching of its fluorescence. This property enabled development of a novel competition-based fluorescent indicator displacement (FID) assay format to quantify MIF binding. In addition, we demonstrated that the 7-hydroxycoumarin also interfered with MIF activities on the cellular level. Therefore, we provide a novel FID assay to study MIF binding and a potent inhibitor for MIF induced signaling in cellular assays to advance research on MIF as potential drug target.



DEVELOPMENT OF KINASE INHIBITORS FOR THE TREATMENT OF GLIOMA VIA A PHENOTYPIC-DRUG DISCOVERY APPROACH

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Glioblastoma multiforme (GBM) is the most aggressive of all brain tumours, with the average survival post diagnosis at, on average, 12 months. Even with maximum available treatment, which is currently resection, followed by radio- and chemotherapy this is only extended to 14 months. Given the poor clinical outcomes for this cancer, this represents a significant clinical need. Genomic characterisation of this cancer has shown that receptor tyrosine kinases are disrupted, as such a kinase inhibitor could be used to treat glioma.[1]

Given the complex genomic aberrations present in glioma, a traditional target-based approach may not be sufficient to discover the best potential alternative therapeutics. Ligand-based phenotypic-drug-discovery had been used to design and synthesise a range of novel molecules for the treatment of GBM, which have been screened in phenotypic assays.[2,3]

A range of novel inhibitors were designed around an ATP-based core to probe structure-activity-relationships of the scaffold. Data collected from the phenotypic screens, carried out in U87 and T98 cell-lines has been used to drive three subsequent rounds of drug design and screening through the analysis of emerging structure-activity relationships. By designing and screening in this iterative manner, several lead molecules with near sub-micromolar potency have been discovered.

To date, over 100 novel molecules have been designed and synthesised. Through screening, hit and lead compounds have been found to have EC50's in the low μM and nm range. Lead molecules have been further screened in two patient-derived cell-lines, grown in both 2D and 3D cell-culture conditions, and a blood-brain-barrier cell model, to determine their behaviour in a range of different cancerous and healthy cells. This data has been used to identify two key molecules for further rounds of optimisation and screening prior to target deconvolution studies to determine their modes-of-action and inhibitory profiles.

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MOLECULAR DOCKING AND COMPUTATIONAL STUDIES AS A TOOL FOR A QUICK SCREENING ON DRUG INHIBITORS FOR SARS-COV-2 RNA-DEPENDENT POLYMERASE

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In silico molecular docking took place in order to evaluate already FDA approved drugs for several diseases, as inhibitors for SARS-Cov-2 RNA- dependent polymerase. The best candidates with the highest binding affinities, evaluated for their energy determined by their electron density. Remdesivir and Saquinavir seemed to be good candidates for clinical trials but their predicted toxicity based on their calculated structures revealed that these two substances should evaluated more for their side effects. We did this theoretically, with much less time and for the first time in our knowledge we make the use of DFT studies on the best candidates in order to find similarities on structures of that molecules that makes them good inhibitor candidates for nsp12. This knowledge, in correlation with the theoretical structural evaluation gave as valuable information about the toxicity of those candidates, a fact that should be studied further.

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DEVELOPMENT OF POTENTIAL HIV-1 NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTIs) BASED ON PRIVILEGED INDOLE SCAFFOLD

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HIV type 1 (HIV-1) reverse transcriptase (RT) is an enzyme who catalyzes the conversion of viral RNA into DNA, initiating a process leading to integration of proviral DNA into the host genome [1]. This reaction is a central step in viral replication, thus it is considered an important target for anti-HIV therapy [2]. In this study, focused on the design and synthesis of RT inhibitors, a new scaffold of potential leader molecules was designed upon the bases of known typical characteristics of NNRTIs: 2 hydrophobic wings attached to a hydrophilic body [3]. Investigation involved several computational screening methods which were applied in a series of **1**-**4** propanamides derived from β^2-tryptophan, that incorporate into its structure a homocyclic (**1**-**3**) or heterocyclic (**4**) hydrophobic aromatic group, including its synthesis and experimental tests. Computed protein-ligand binding energy and *in vitro* inhibitory activity were compared with those of medicinal compounds delavirdine® and efavirenz®. It was shown that although propanamides **1**-**4** theoretically bind at the allosteric site of RT (PDB: 1KLM), with a ΔG of about -10 Kcal/mol (Table), they do not inhibit RT *in vitro*. However, the structural motif of propanamide continues being a perspective in terms of design of a new chemical class of NNRTIs.

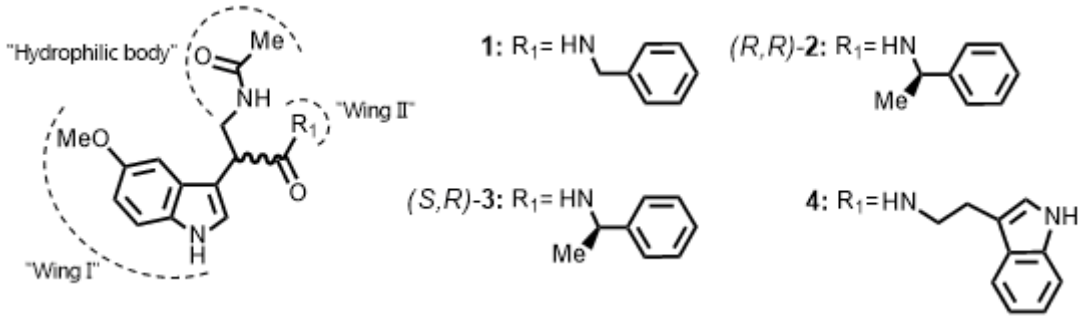

Table. Binding affinity (ΔG, Kcal/mol) of propanamides **1**-**4** and delavirdine (**DLV**) in complex with RT.

Ligand	1	(*R*,*R*)-2	(*S*,*R*)-3	4	DLV
ΔG	-9.87	-10.26	-10.21	-10.90	-13.04

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DETERMINATION OF TRANSITION METAL ION IMPURITIES IN CYCLOTRON PRODUCED 44Sc AND 68Ga BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

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PET (Positron Emission Tomography) imaging provides functional information about living systems with the detection of gamma photons (511 keV). The labeling of antibodies, peptides and proteins with radiometals can be more effective than the application of ^{18}F or ^{11}C. Scandium-44 (^{44}Sc) and Gallium-68 (^{68}Ga) decays with positron emission and has good PET imaging properties. One of the many advantages of these radionuclides is the rapid radiolabeling with appropriate chelators. ^{44}Sc and ^{68}Ga can be produced by cyclotron from the proton irradiation of appropriate metal targets ($^{44}Ca(p,n)^{44}Sc$[1]; $^{68}Zn(p,n)^{68}Ga$[2]).

The cyclotron produced radiometals must be separated from the irradiated target and purified from other metal impurities[3,4],which could influence the yield of the radio-labeling process. DOTA chelator was used as model compound to test the purity and reactivity of the produced metal isotopes. The results of the labeling reactions were followed by RadioTLC. The radionuclidic contaminants were determined by gamma spectrometry, while the quantitave determination of inactive metals were carried out by ion chromatography.
4-(2-pyridylazo)resorcinol (PAR) is an unselective chelating agent, forming water-soluble chelates with most of the transition metals immediately at room temperature[5]. PAR was added to the the samples before injection. The light absorbing complexes of the examined metals were separated using a reversed-phase analytical column (Lichrospher RP18). The separation took 10 minutes, the UV chromatograms were integrated at 530 nm and the measurements were carried out with a flow rate of 0.8 mL/min. The mobile phase was 65 % 0.1 M pH 6.5 $NH_4H_2PO_4/(NH)_4HPO_4$ buffer and 35 % methanol. The metal contaminants including Co^{2+}, Fe^{2+}, Cu^{2+} and Ni^{2+} were also analyzed by using this method. The limit of quantitation for all metal ions were in the ppm range.

The developed chromatographic method can be used in the quality control of cyclotron produced rradiometals. The limit for iron content in cyclotron produced ^{68}Ga was defined to be 10 ppm/GBq[6]. The present method is suitable to quantify iron and other mentioned transition metals in this concentration range.

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LIPID TAGGED TEMPORIN L-DERIVED PEPTIDES: AN AVENUE TO FIGHT ANTIBIOTIC RESISTANCE

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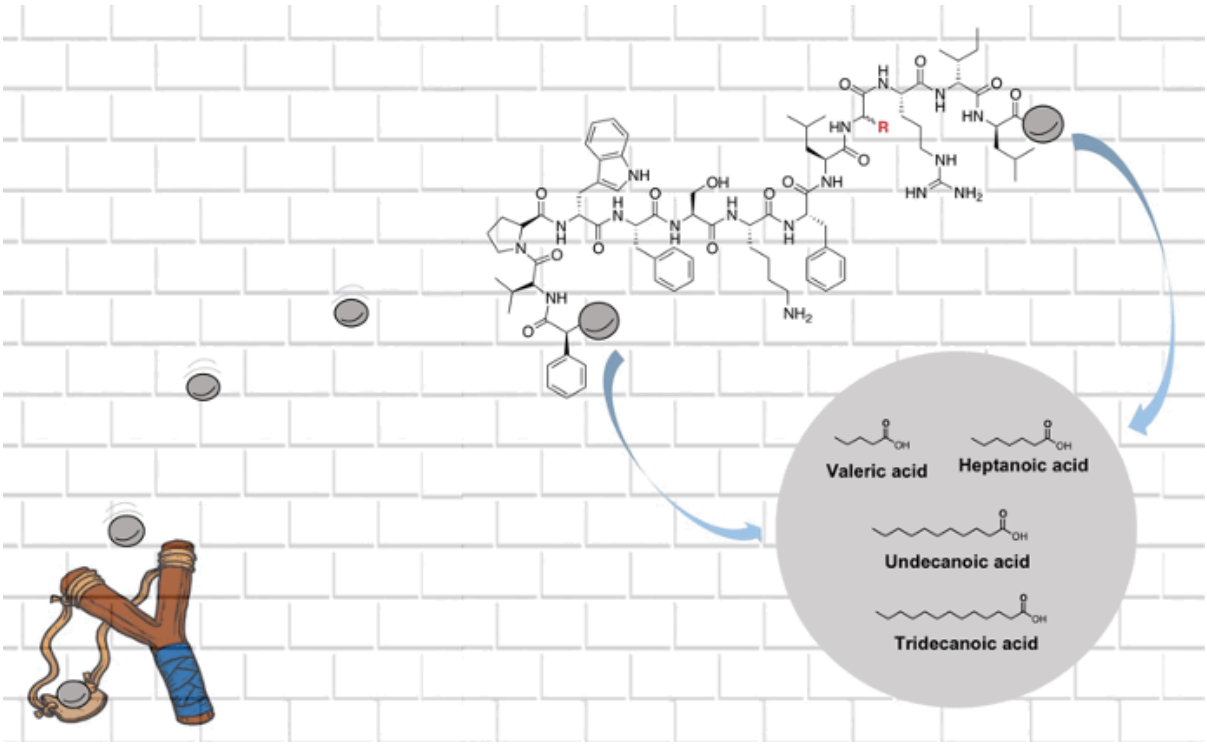
Antimicrobial peptides (AMPs) represent a valid chance to overcome and control the antibiotic resistance, since they act by a different mechanism of action from conventional antibiotics. Temporins represent one of the largest families of AMPs.[1] We designed and synthesized Temporin L (TL) analogues by introducing fatty acids of variable length as chemical motif (tag) in both N- and C-terminal (Figure 1). Such modification lends to peptides an increase of hydrophobicity and incorporation in lipid bilayers, influencing effectively their antimicrobial activity.[2][Insert Image tag]



The antimicrobial activities of all peptides were evaluated both on *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella Pneumoniae* ATCC BAA-1705 and on clinically isolated strains. The self-assembling of peptides was determined by critical aggregation concentration, their mechanism of action was investigated by performing fluorescence assays (Laurdan, Thioflavin T and Membrane Leakage assays) and finally their biostability in human serum was assessed.

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PHOTOSWITCHABLE ANTAGONISTS FOR A PRECISE SPATIOTEMPORAL CONTROL OF β2-ADRENOCEPTORS

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β_2-Adrenoceptors (β_2-AR) are prototypical G-protein-coupled receptors and important pharmacological targets. Indeed, a number of approved drugs target these receptors due to their key role on many important physiological functions.[1,2] Therefore and considering the therapeutic value of these receptors, achieving a reversible and localised control of their activity would provide a powerful tool, both for its research applications and its clinical potential. In this context, photopharmacology arises as a potent approach.[3]

Herein, we introduce **Photoazolol-1–3**, a series of photoswitchable azobenzene β_2-AR antagonists that can be reversibly controlled with light.[4] These new photochromic ligands are designed following the azologization strategy, with a *p*-acetamido azobenzene substituting the hydrophobic moiety present in many β_2-AR antagonists. Using a fluorescence resonance energy transfer (FRET) biosensor-based assay, a variety of photopharmacological properties are identified. Two of the light-regulated molecules show potent β_2-AR antagonism and enable a reversible and dynamic control of cellular receptor activity with light. Their photopharmacological properties are opposite, with **Photoazolol-1** (**PZL-1**) being more active in the dark and **Photoazolol-2** (**PZL-2**) demonstrating higher antagonism upon illumination. In addition, we provide a molecular rationale for the interaction of the different photoisomers with the receptor. Overall, we present innovative tools and a proof of concept for the precise control of β_2-AR by means of light.

[image]

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SURFACE-ACTIVE IONIC LIQUIDS DERIVED FROM ANTIMALARIAL DRUGS AND NATURAL FATTY ACIDS

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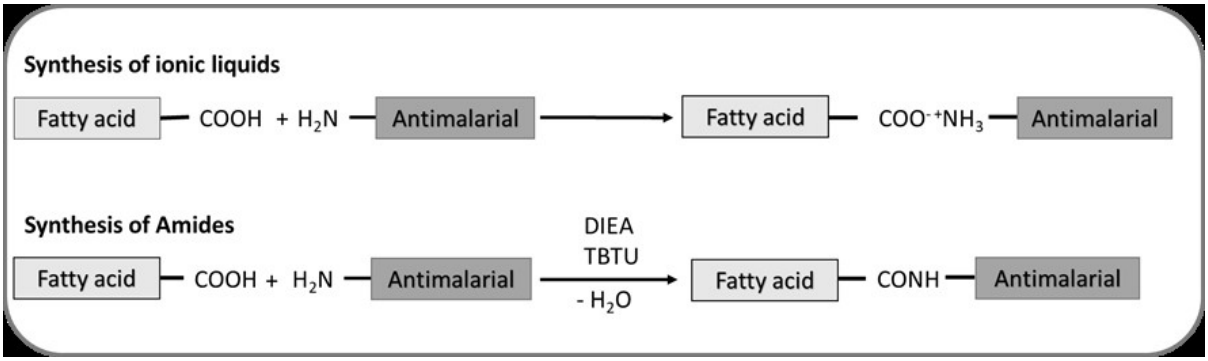
The use of Ionic Liquids in Medicinal Chemistry is becoming increasingly popular, [1] and could represent a new cost-effective approach to rescue old drugs, like primaquine and chloroquine, in the fight against malaria. As such, and through two simple reactions [2][3], we synthesized ionic and covalent conjugates of the aforementioned antimalarial drugs with natural fatty acids (Scheme 1).



The thermal stability of the compounds was evaluated by simultaneous thermal analysis (STA), revealing that both the ionic liquids and the amides are stable to thermal degradation up to 90 ºC or above. Also, considering the amphiphilic nature of fatty acid salts, the interfacial properties of the new ionic liquids was investigated. It was found that the compounds are able to form mixed micelles with a cationic surfactant (CTAB) and that there is a certain fatty acid chain size that is particularly effective in decreasing the critical micelle concentration of the mixture. Finally, biological activity of all compounds was assessed in vitro both on liver- and blood-stages of malaria, which showed that (i) all compounds are active; (ii) amides are better than the ionic liquids against liver-stage parasites, but are significantly more insoluble; (iii) the ionic liquids stand out as potent blood-stage parasites, and (iv) there is an optimal fatty acid chain size for the display of this activity.

In conclusion, we disclose novel surface-active ionic liquids whose antimalarial activity in vitro is superior to that of the parent drug. These findings open new perspectives for and beyond malaria chemotherapy.

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IN SILICO FRAGMENT-BASED STUDIES OF SPIROOXINDOLES AS INHIBITORS OF BETA-SECRETASE (BACE1)

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Abstract: Alzheimer's disease (AD) is the most common type of neurodegenerative disorder, mainly in older ages. It is responsible for around 60-70% of dementia cases worldwide, affecting more than 30 million people[1]. One hallmark of this disease is the presence of extracellular amyloid "plaques", composed primarily of proteolytic fragments of amyloid precursor protein (APP). In the past 20 years, intense research has focused on creating inhibitors of β-secretase (BACE1) -a transmembrane aspartyl protease that cleaves APP at the β-site[2]- as an important strategy for pharmacological intervention in AD[3]. The aim of this study is to build drug-like ligands as inhibitors against BACE1 starting from small fragments and to accurately predict their binding modes (FIgure 1).

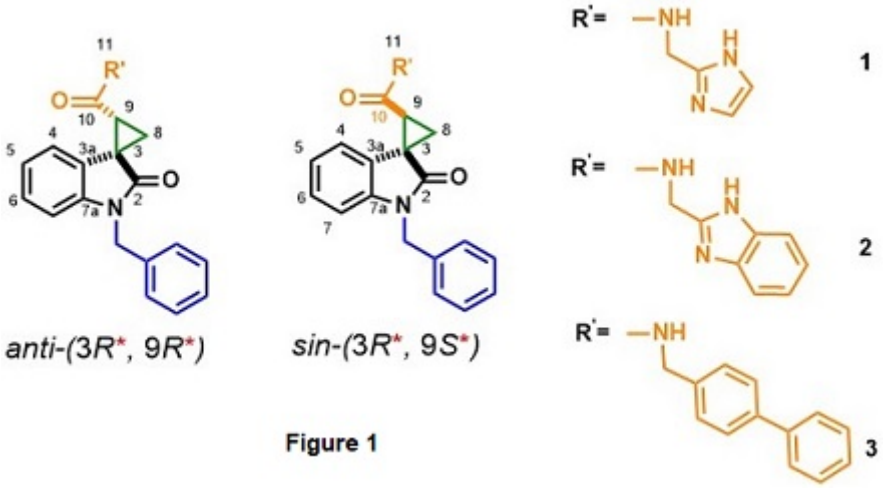

Figure 1

Methods and Results: A computational docking study (AutoDock 4) was performed using the enzyme BACE1 (PDB: 3UDK). The results show that all the proposed ligands **1**-**3** interact within the catalytic site of the enzyme, with a binding affinity (ΔG, Kcal/mol) of -7.78, -8.13 and -9.50, respectively. In particular, the complex BACE1-**3**, with the best energy value (ΔG = -9.50), is stabilized by two hydrogen bonds with Asp 32 and Tyr 71. In addition, the presence of aromatic rings generates important hydrophobic interactions within the surrounding residues of the catalytic site. The synthesis and characterization of the new compounds are currently being carried out.

Conclusion: The use of the fragment-based drug design strategy, accompanied by in-silico studies identified a novel spirooxindole-type compounds with affinity against the BACE1 enzyme.

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EVALUATION OF ANTIMICROBIAL AND WOUND HEALING EFFECT OF METHANOL EXTRACT OF CHASMANTHERA DEPENDENS ROOTS ON WISTAR RATS

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Background: The recent increase in antimicrobial resistance and emergence of resistant species has diverted most recent research attention, not only to identify these species but also to the possible discovery and development of plant-based principles with antimicrobial potentials.

Objectives: To investigate the anti-microbial and wound healing properties of the methanol extract of *Chasmanthera dependens* roots (MECDR) on wistar rats. The phytochemical profile and acute toxicity studies are reported in tandem.

Methods: Phytochemical studies and acute toxicity studies of the extract were carried out following standard methods. The antimicrobial activity of *Chasmanthera dependens* against six bacteria species was analyzed *in vitro* using the agar well diffusion technique. The zones of inhibition produced by different concentrations of the extract after 24 hours of incubation were compared to that of Ciprofloxacin. The minimum inhibitory concentration of the extract was assessed through macro broth dilution technique. To evaluate the wound healing activities of the extract, wound-induced rats were treated with different doses of the extract and Ampiclox and the wound length measured for 12 consecutive days.

Results: MECDR was found to be safe at a maximum dose of 5000 mg/kg body weight. The phytochemical profile showed the presence of flavonoids, alkaloids, tannins and saponins. MECDR demonstrated a dose-dependent antimicrobial activity with average inhibition zone diameters ranging from 6.75- 19.0mm. At the highest dose of 200 mg/mL, the extract produced largest inhibition zone diameters of 13.0mm, 19.0mm, 13.5mm, 17.0mm, 14.0mm and 17.3mm against *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, Enterococcus sp., Escherichia coli* and *Bacillus sp* respectively, comparable to 36.0mm, 33.0mm, 33.0mm, 39.0mm, 35.0mm, and 33.5mm produced by 250 µg/mL of ciprofloxacin. The mean MICs and MBCs of the extract and drug varied from 6.3- 100 mg/mL and 125- 250 mg/mL respectively. *P. aeruginosa, S. aureus, Enterococcus. sp., E. coli* and *Bacillus sp* sensitive to the extract at 6.3 mg/mL demonstrated a higher inhibitory rate than *K. pneumonia* (12.5mg/mL). MECDR also demonstrated 86.97%, 93.13% and 95.23% wound healing capacity at 200, 400 and 600mg/kg respectively, compared to 87.49% by 500mg/kg of Ampiclox. Therefore, the extract exhibited a significant dose-independent wound healing activity.

Conclusions: This study, demonstrated that MECDR possesses antimicrobial and wound healing properties which could be attributed to the presence of bioactive principles. It is, therefore, a promising raw material for the preparation of safe antimicrobial therapeutics.

Keywords: medicinal plants; *Chasmanthera dependens;* antimicrobials; antimicrobial-resistant strains.

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INVESTIGATING UGI / PASSERINI MULTICOMPONENT REACTIONS FOR THE SITE-SELECTIVE CONJUGATION OF NATIVE TRASTUZUMAB

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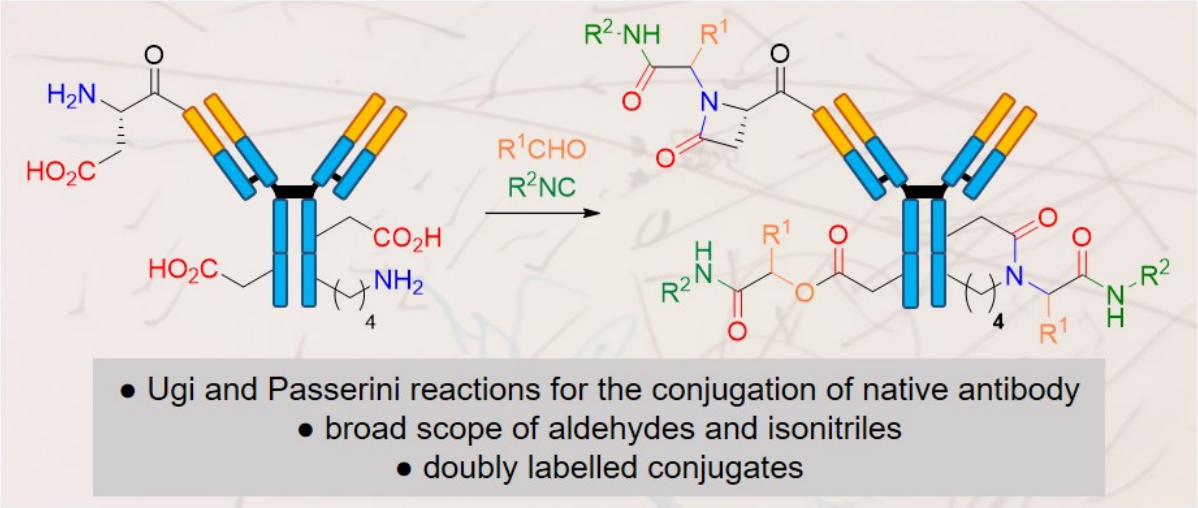
Site-selective modification of proteins has been the object of intense studies over the past decades, especially in the therapeutic field. Prominent results have been obtained with recombinant proteins, for which site-specific conjugation is made possible by the incorporation of particular amino acid residues or peptide sequences.[1-3] In parallel, methods for the site-selective and site-specific conjugation of native and natural proteins are starting to thrive, allowing the controlled functionalization of various types of amino acids residues.[4,5] As the vast majority of protein conjugation strategies focus on the modification of a unique residue, we hypothesized that targeting two different amino acid side chains simultaneously should give higher chance of developing a site-selective strategy. We opted for the Ugi four-center three-component reaction to implement this idea, with the aim of conjugating the side-chain amine and carboxylate groups of two neighboring lysine and aspartate/glutamate. We showed that this strategy can give access to valuable antibody conjugates bearing several different payloads, and limits the potential conjugation sites to only six on the model antibody trastuzumab.



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DEVELOPMENT OF CHEMORESISTANT COLORECTAL CELL LINE

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Multiple drug resistance (MDR) is a serious problem in chemotherapy of proliferative diseases. One of the main mechanisms, upon activation of which the development of resistance to a wide range of drugs is observed, is an increased expression of ABC transporters, transmembrane transport proteins. The main role in the emergence of multidrug resistance is played by multidrug resistance protein 1 (MDR1, P-glycoprotein), multidrug resistance-associated protein 1 (MRP1), and breast cancer resistance protein (BCRP) [1]. In healthy cells, ABC transporters provide the transfer of needed compounds, and also release xenobiotics, protecting the cell from intoxication. In tumor cells, transport proteins provide the release of drugs and provoke the development of chemoresistance.

The development of anticancer drugs capable of overcoming MDR is an important issue of modern oncology. In order to study the activity of transporters and the effect of potential inhibitors on it, the development of resistant cell lines based on human colon cancer cell line HCT116 is carried out in the Laboratory of Molecular Pharmacology. For this, wild-type HCT116 cells are cultured in a medium with anticancer drugs of various mechanisms of action, Paclitaxel and Nutlin-3a, under gradual increase in drug concentration.

In parallel, selection of HCT116 cells for their ability to absorb Rhodamine 123 is carried out. This fluorescent dye is a substrate for P-glycoprotein, and it can be expected that a less intense accumulation of the dye in cells corresponds to a greater activity of the transporter. Cells that are weakly stained with Rhodamine 123 are selected using the high content analysis system (Operetta CLS ™) after 40 min of incubation with the dye.

Resistance (including cross-resistance), as well as expression levels of transport proteins, will be studied for the strains obtained.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW METHOXY NAPHTHALENE DERIVATIVES AS POTENTIEL ANTICANCER AGENTS ON MCF-7 BREAST CANCER CELLS

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According to the World Health Organization, cancer is responsible for approximatively 9.6 million death worldwide and its incidence is still increasing as 18.1 million of new cases were collected in 2018. [1] Breast cancer is the most frequently diagnosed and its incidence rate far exceeds those of other cancers in both developed and developing countries. [2] Chemotherapy is one of the major treatments proposed for cancer and a number of anticancer drugs are currently in use. However, critical drawbacks and development of multi-drug resistance (MDR) are often associated with these drugs and led to poor therapeutic efficiency. So, there is still a demand for new drugs with improved potency and lower toxicity and continuous efforts to find new anti-cancer agents are made in both academic and industrial settings. [3] Natural products represent a valuable and promising source for the discovery of novel lead drugs and some oncology medicines are derived from plants. Therefore, synthetic modifications of natural products for elaboration of new drug still remain an important goal .

The recent report about the cytotoxic potential on various cancer cells lines [4] of Guieranone A, [5] compound isolated from leaves of *Gueira Senegalensis,* [6] prompt us to explore the synthesis of analogues with increased potency. A series of methoxy naphthalene derivatives were prepared in good overall yields, through the naphthol route and all compounds were evaluated for their antiproliferative activity against MCF-7 cell line. The most of compounds displayed potent antiproliferative activity. Among them, compound **EMOP-157** displayed the most potent antiproliferative activity with an IC_{50} value of **3.23** µM, as compared to 5-FU (IC_{50} **4.4** uM) et tamoxifène (IC_{50} **11,91** uM).

Mots clés: *Méthoxy naphtalène, MCF-7, breast cancer, guiéranone A, Gueira Senegalensis*

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DRUGS AND MUCUS: AN INNOVATIVE BIOSIMILAR MUCUS MODEL TO STUDY THE DIFFUSION OF DRUGS

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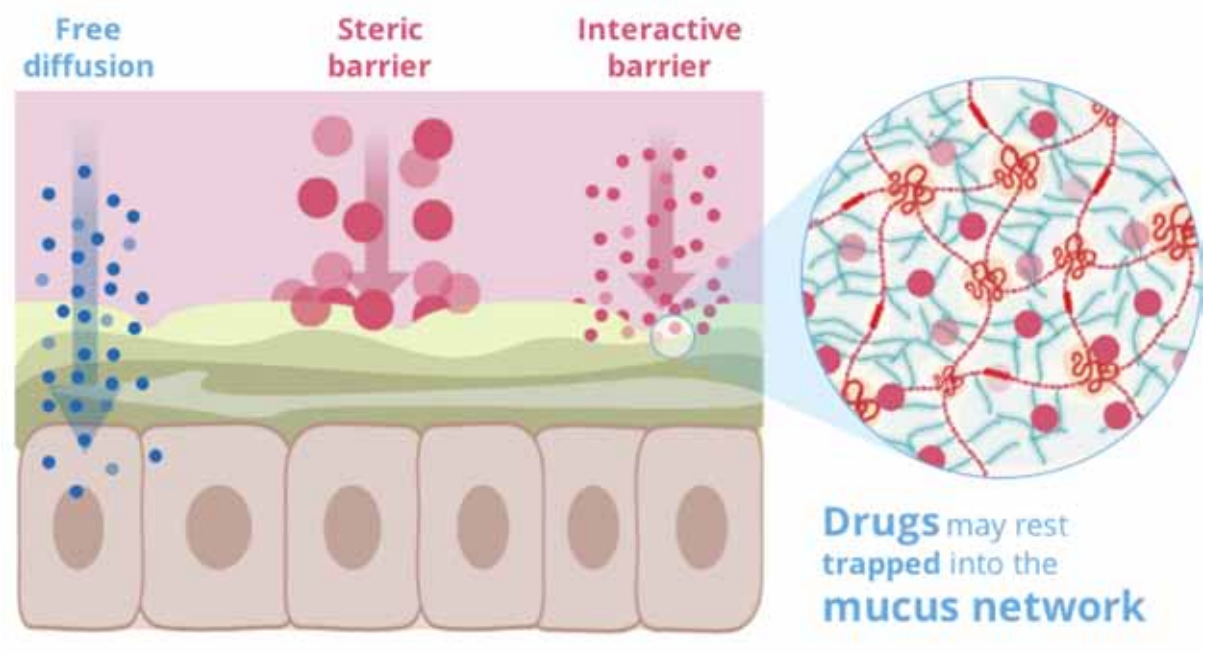
Background. A thin layer of mucus covers all the wet epithelia of the human body ensuring protection against exogenous compounds. Orally or pulmonary administered drugs have to overcome the mucosal layer in order to be absorbed and then to be effective (Fig. 1). However, mucus can represent a strong barrier to overcome even for drugs. Despite the crucial role played by mucus on drug absorption, there are no standard mucus models to be employed in the early drug discovery process for the screening of potential drug candidates [1].

Methods. We have developed a biosimilar mucus model that mimics a pathological mucus. The viscoelastic behavior of mucus was recreated by using a natural polysaccharide while the composition was mimicked by adding mucin, the main glycoprotein forming mucus. By coupling the biosimilar mucus model to the state-of-art permeability platforms (i.e. parallel artificial membrane permeability assay), the diffusion of some drugs through both mucus and cellular membrane, was investigated. The amount of diffused drug was quantified and reported as percent of total.

Results. The herein presented mucus model was able to discriminate between the mucin-drug interaction and the steric barrier of a mucus layer with respect to PAMPA test [2, 3].

Conclusion. Since the drug development is characterized by a high rate of failure, the mucus platform could help to reduce the number of non-effective drugs that reach the preclinical trials. Moreover, the model is completely tunable as the production method allows to easily include other molecules present within mucus (lipids, DNA, proteins).

Keywords: mucus, hydrogel, mucin, drugs, diffusion



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ENONE AND AZOMETHINE MOIETIES TO TARGET INFLAMMATION

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Inflammation is a complex phenomenon that results as a healing response of organisms to different factors, exerting immune signaling, excessive free radical activity and tissue destruction. It is believed to be involved in numerous diseases such as cancer, and senile dementia Alzheimer's type. Lipoxygenase (LOX) and Cycloxygenase (COX) pathways play an important role in inflammatory sites in correlation with the reactive oxygen species (ROS) produced during the inflammation by phagocytic leukocytes. ROS are involved in the LOX and COX mediated conversion of arachidonic acid into pro-inflammatory intermediates.

The aim of our study is to synthesize compounds which include enone and azomethine moieties. In particular, the first group of compounds combine the cinnamic and curcumin pharmacophore (cinnamic hybrids), while the second group are hybrids of thiosemicarbazones.

Computer-aided drug design was used for the candidates' synthesis selection, which was partly based on published procedures. The cinnamic and thiosemicarbazone hybrids play a vital role in the formation of commercially important intermediate molecules which are necessary for the production of different bioactive compounds and drugs. Cinnamic acid hybrids present a wide range of biological activities: antituberculosis, antidiabetic, antioxidant, antimicrobial, hepatoprotective, central nervous system stimulant (CNS), antidepressant, anticholesterolemic, antiviral, anxiolytic, cytotoxic and anti-inflammatory. Thiosemicarbazone hybrids: antioxidant, anti-proliferative and anti-mycobacterium.

Furthermore, the combination of appropriate pharmacophore groups led to conjugates with multi-target activities. In recent years, intensive research on hybrids has been conducted in order to create new multifunctional drugs. The results of our synthetic efforts are several groups of newly synthesized compounds that were subjected to further optimization. The new derivatives were characterized based on the structural characteristics and physicochemical properties of the molecules. Preliminary antioxidant inhibitory activity in vitro tests have been performed followed by inhibition of soybean LOX and COX. The physicochemical properties of the compounds were analyzed in terms of Lipinski's rule. Further investigation is in progress concerning their multi-target profile.

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF 2-OXOPYRIDINE-3-CARBOXAMIDE DERIVATIVES AS POSITIVE ALLOSTERIC MODULATORS OF CANNABINOID TYPE-2 RECEPTOR

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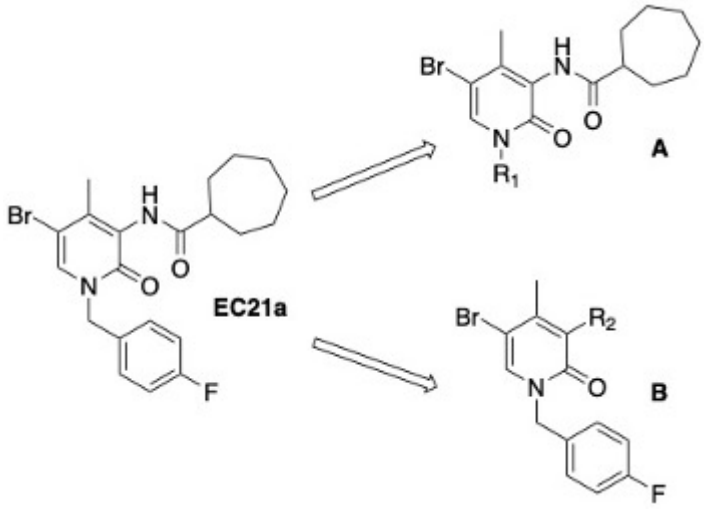
The endocannabinoids (ECs) are neuromodulatory signaling molecules implicated in a large range of biological processes including cognition, analgesia, stress, anxiety, neuroprotection and motor coordination. Arachidonoyl ethanolamide (anandamide or AEA) and 2-arachidonoyl glycerol (2-AG) are the most important ECs and both are synthesized on demand from membrane phospholipids and function through the G-protein coupled cannabinoid receptors (CBRs CB1R and CB2R). Since the CBRs discovery, many efforts have carried out to produce highly selective orthosteric compounds that would be suitable as potential therapeutic agents. In many cases, direct-acting agonists have proved to lead to serious adverse effects, such as anxiety, depression, suicidal ideation, psychotropic effects or immune dysfunction, that have limited the clinical development of classical orthosteric ligands. For that, the interest in the allosteric regulation as new way to modulate the receptor activity without the drawbacks associated with the direct agonist activation, has been steadily increasing in the CBRs field. In fact, by binding at sites that are topologically and often functionally distinct from their orthosteric counterparts, allosteric ligands typically afford high levels of selectivity.
The great interest directed to the discovery of CBRs allosteric ligands has recently led to the identification by our research group of the compound **EC21a** as the first synthetic positive CB2R allosteric modulator [(a)].
In the present study, structural optimization of a previously developed 2-oxopyridine-3-carboxamide class, to which **EC21a** belongs, led to the newly synthesized derivatives of general structure **A** and **B** (Figure 1).



. Figure 1. Compound **EC21a** and general structure of the new derivatives **A** and **B**.

The new compounds have been tested *in vitro* through radioligand binding experiments and functional assays (cAMP, βarrestin2, GTPγS). In particular, two **A**-series compounds showed to be novel positive allosteric modulators of cannabinoid type-2 receptor, similarly to the parent compound **EC21a**. The results reported in this work might be useful for the characterization of the CB2R's allosteric site which has been recently postulated to exist adjacent to the CB2R orthosteric binding site.

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SYNTHESIS AND EVALUATION OF ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF 1,3-DIHYDROXYXANTHONE AND ITS DERIVATIVES

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Abstract
Alzheimer's disease (AD) is a neurological disorder resulting in the progressive and irreversible cognitive impairment which eventually leads to disabilities and life-threatening conditions. The current treatment for AD includes cholinesterase inhibitors, which aims to increase the bioavailability of acetylcholine and enhance brain neurotransmission. Unfortunately, the currently marketed drugs are associated with adverse effects including gastrointestinal disorders, confusion, fainting, and acute hepatocellular toxicity. Therefore, this study aims to explore possible novel lead compounds for AD treatment, which involves syntheses of a class of bioactive compound, xanthone and its derivatives, and their acetylcholinesterase (AChE) inhibition activity. The parent compound, 1,3-dihydroxyxanthone, and a total of nineteen new derivatives consisted of alkyl, alkenyl, alkynyl and alkylbenzenes substituent groups were synthesized. The compounds were purified by chromatographic techniques and structurally characterized and elucidated by spectroscopic methods including FTIR, GC-MS, and NMR. The AChE inhibition activities of the synthesized compounds were evaluated by Ellman's assay and the results showed that the derivatives exhibited moderate to strong inhibition with IC_{50} values at a micromolar range of 20.8-71.2 μM. In particular, (3-(but-3-en-1-yloxy))- and (3-(pent-4-en-1-yloxy))-1-hydroxy-*9H* -xanthen-9-one displayed the strongest AChE inhibitory activities. The AChE enzyme kinetic experiments conducted on these derivatives have shown mixed inhibition mode of competitive and non-competitive inhibition. The structure-activity relationship (SAR) study revealed that the xanthone with alkenyl substituent groups contributes to a stronger anti-AChE effect. The study concluded that these xanthone derivatives possess good anti-AChE properties and potentially further developed into anti-AD drug(s). Further molecular docking, ADME-toxicity studies on the alkenyl-substituted xanthone derivatives are recommended to visualize their molecular binding interactions and assess their pharmacokinetic properties.

Keywords
Alzheimer's Disease, Structural Modification, Enzyme Kinetics, Ellman's Assay

Acknowledgement
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NEW MARINE-DERIVED INDOLYMETHYL PYRAZINOQUINAZOLINE ALKALOIDS WITH PROMISING ANTIMICROBIAL PROFILES

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Due to the emergence of multidrug-resistant pathogenic microorganisms, the search for novel antimicrobials is urgent. Inspired from marine alkaloids, a series of indolomethyl pyrazino [1,2- b]quinazoline-3,6-diones was prepared using a one-pot microwave-assisted multicomponent polycondensation of amino acids. Compounds were evaluated for their antimicrobial activity against a panel of nine bacterial strains and five fungal strains. Compounds 26 and 27 were the most effective against Staphylococcus aureus ATCC 29213 reference strain with MIC values of 4 μg/mL, and a methicillin-resistant Staphylococcus aureus (MRSA) isolate with MIC values of 8 μg/mL. It was possible to infer that enantiomer (-)-26 was responsible for the antibacterial activity (MIC 4 μg/mL) while (+)-26 had no activity. Furthermore, compound (-)-26 was able to impair S. aureus biofilm production and no significant cytotoxicity towards differentiated and non-differentiated SH-SY5Y cells was observed. Compounds 26, 28, and 29 showed a weak antifungal activity against Trichophyton rubrum clinical isolate with MIC 128 μg/mL and presented a synergistic effect with fluconazole. In conclusion, simpler marine-inspired indolomethyl pyrazino [1,2-b]quinazoline-3,6-diones derivatives were discovered as promising antimicrobial agents.

Acknowledgements: This research was supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020, UIDP/04423/2020, and under the project PTDC/SAU-PUB/28736/2017 (reference POCI-01-0145-FEDER-028736), co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF and by FCT through national funds.

SYNTHESIS OF NEW 3-O-SUBSTITUTED XANTHONE DERIVATIVES: CHOLINESTERASE ENZYME INHIBITORY ACTIVITIES AND MOLECULAR DOCKING STUDY

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Alzheimer disease (AD) accounted for 60 to 80% of all cases of dementia, besides Lewy body dementia, frontotemporal disorders and vascular dementia. Limited market available effective drugs and the side effects that are associated with these drugs increased the demand to search for potential lead compounds that possess significant anti-Alzheimer's diseases activities. Most of the clinical drugs are derived from natural products and their derivatives. Xanthone, for instance, is an important plant secondary metabolite with wide range of biological activities and thus potential to be developed as a cholinesterase inhibitor. In present study, a series of twenty-nine new 3-*O*-substituted xanthone derivatives consisted of alkyl, alkenyl, alkynyl, phenyl, hydroxyl, methoxyl and ethoxyl substituents were synthesized from 3-hydroxyxanthone, characterized and evaluated for their anti-cholinergic activities against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). These xanthone derivatives possessed potent AChE inhibitory activity with IC_{50} values in the range of 0.88-2.39 μM while weaker inhibition towards BChE. The AChE enzyme kinetic study of the most potent inhibitor, 3-(4-phenylbutoxy)-9*H*-xanthen-9-one on AChE showed mixed mode inhibition mechanism. Molecular docking study was performed on this compound to gain further insight of the intermolecular interactions with AChE. The results showed that it bound to the active site and interacts *via* extensive π-π and hydrogen bonding interaction. This study revealed that 3-*O*-alkoxyl substituted xanthone derivatives are potential lead structures and future studies on the molecular mechanism of this derivative is highly recommended.

Keywords: Acetylcholinesterase, Alzheimer's disease, Hydrophobic Substituents, Kinetic study, *O*-Alkylation

Acknowledgement

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PROSPECTIVE VIRTUAL SCREENING OF NATURAL PRODUCTS AS POTENTIAL SARS-COV-2 MPRO INHIBITORS

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By the end of 2019, the new coronavirus identified as COVID-19 spread all over the world, being responsible for causing a worldwide pandemic. This virus infection leads to severe acute respiratory syndrome (SARS-CoV-2) and it may have caused ca. 730,000 deaths until today[1]. Although there is still no effective drug directed to COVID-19 treatment, many efforts have been conducted to find out new potential therapeutics, specially by targeting at the main protease (Mpro), which is an enzyme responsible for such virus replication[2]. Moreover, most of investigations have employed known drugs databases in repurposing strategies. Few studies, however, have explored the diversity of natural products to achieve novel chemical scaffolds in this context as well as the potential of phytotherapeutic agents. Therefore, with this in mind, we performed a prospective ligand-based virtual screening using five natural products databases. We used Chembridge, IBS, Molport, NPASS and ZINC natural products subcollections, consisting of ca. 388,000 molecules, preprocessed by FILTER and OMEGA, and these were filtered by 3D shape similarity using ROCS[3], and using the reference ligand complexed with SARS-Cov-2 Mpro, PDB ID 6W63, recently deposited[4]. Worth mentioning that previous validation of ROCS query was performed - using a dataset of 37 known bioactive compounds towards SARS-CoV-2 Mpro, retrieved from literature, and summed with corresponding decoys generated by DUD-E - by ROC curve analyses, with an AUC = 0.801 (Fig.1a). Furthermore, we also filtered compounds using EON, followed by ADME/Tox evaluation using Qikprop and DEREK, which retrieved less than 100 compounds that were subsequently submitted to docking simulations using Glide. Finally, we obtained 10 potential hits, with interesting chemical diversity and promising biological activity, according to interactions shown by them within SARS-CoV-2 Mpro binding site (Fig.1b). These molecules will be experimentally validated by ongoing in vitro assays and shall become potential natural products-like drug candidates with interest in COVID-19 treatment.

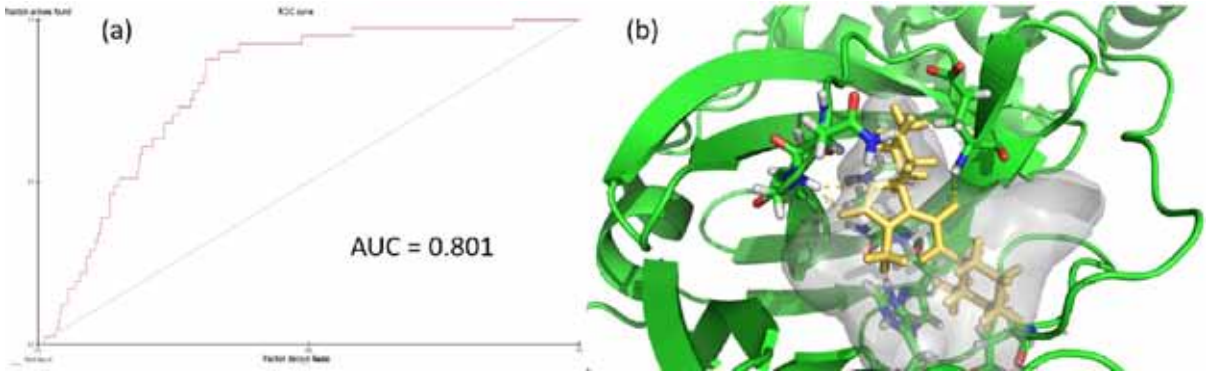

Figure 1. (a) ROC curve built for assessing the performance of ROCS query towards a dataset of 37 known SARS-CoV-2 Mpro inhibitors and DUD-E decoys. (b) Representation of docking pose of an example of 10 potential hits and corresponding intermolecular interactions, obtained by Glide and using SARS-CoV-2 Mpro (6W63.pdb).

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A RATIONAL MERGING STRATEGY TO TURN DONEPEZIL INTO A MULTI-TARGET-DIRECTED LIGAND

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According to WHO data, dementia is currently affecting about 10 million people in Europe, and its prevalence is expected to double by 2030.[1] Unfortunately, there is still no effective treatment against Alzheimer's disease (AD) or any kind of dementia.[1] For this reason, drug discovery in AD represents, not only the major challenge of the 21st century, but also an urgent medical need. The most promising strategy to face the multifactorial nature of AD seems to be the polypharmacology by multi-target-directed ligands (MTDLs).[2] On these bases, and considering the evidence of synergic effects provided by the combination of donepezil (**1**), a drug currently available on the market to treat AD, and quinone drug idebenone (**2**), we manipulate the structure of **1** to obtain a MTDL small library **3**-**15** (Figure 1).[3] Thus, aiming to enrich **1**'s cholinesterase inhibitory effect introducing anti-amyloid and antioxidant activities we replaced the indanone scaffold with the bioisosteric 1,4-naphthoquinone (Figure 1).[3]

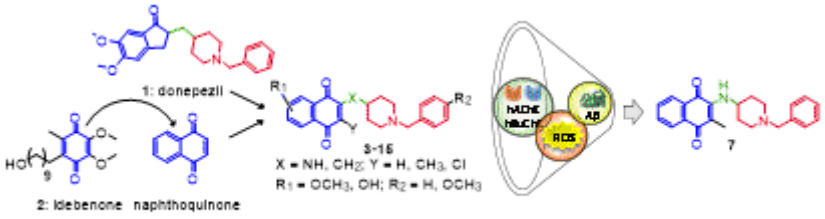

Figure 1: Rational design of novel 1-based MTDL library and biological assays.

Differently substituted 1,4-naphthoquinones have been selected and linked to the benzylpiperidine moiety by CH_2 or NH bound. N-linked compounds were synthesized through classical SN_2 nucleophilic substitution reactions of 2-haloderivatives with amines, or direct 1,4-type addition of amines, while the CH2-linked target compound was obtained by a radical reaction between the naphthoquinone moiety and the piperidine fragment.[3] In order to characterize the multi-target profile of **3**-**15**, compounds were tested for their ability to inhibit human acetylcholinesterase (hAChE) and butyrylcholinesterase (hBuChE) by Ellman's assay. Regarding hAChE inhibition, all compounds showed less activity than **1** conversely, the results on the anti-BuChE activity were more encouraging and disclosed **7** (IC_{50}: 95.7 nM, hAChE SI: 0.002) and **13** (IC_{50}: 0.795 μM, hAChE SI: <0.008) as good and selective hBuChE inhibitors. Combining potency and selectivity data from AChE/BuChE enzymatic screening, hits **5**-**7** and **13** were selected for further assays.[3] They demonstrated positive permeability values at PAMPA-BBB assay, and were screened for their cytotoxicity and neuroprotective profile. Despite the insertion of a quinone fragment, **5**-**7** and **13** retained the favorable hepatotoxicity profile of **1** in Hep-G2 cells. When assayed in in SH-SY5Y cells, **6** showed a dose-dependent decrease of viability , while **5**, **7** and **13** displayed a quite safe neurotoxicity profile.[3] Because of the toxicity issue of **6,** only **5**, **7** and **13** were progressed to neuroprotection studies against oxidative stress. The antioxidant activity was evaluated in SH-SY5Y cells in the absence and presence of *t*-BuOOH, to induce oxidative stress. Experiments were also performed with cells treated with sulforaphane, an inducer of NQO1 enzyme, to exert whether the antioxidant potential of **5**, **7** and **13** generating the corresponding hydroquinones. Finally the most promising compound **7** was evaluated for its antiaggregating properties against Aβ42 self-aggregation by thioflavin T fluorescence assay.[3] Conversely to **1**, derivative **7** significantly inhibited Aβ42 self-aggregation (52.0 ± 8.0 % inhibition, at 1:1 ratio with amyloid).[3] Finally, to get further proof of its drug-likeness, **7** safety was evaluated on a primary cell line of cerebellar granule neurons. We can conclude that **7** can represent an example of a first line symptomatic AD medication converted in a promising MTDL hit with a potential disease-modifying profile.[3]

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IN SILICO AND BIOPHYSICAL APPROACHES TO SHED LIGHT ON MOLECULAR RECOGNITION OF PD-1/PD-L1 INHIBITORS

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The programmed death-1 molecule (PD-1) and its ligand (PD-L1) are members of the CD28/B7 superfamily. This protein-protein interaction is involved in the regulation of T cells response and the maintenance of peripheral tolerance. Therefore, blocking the PD-1/PD-L1 pathway has proven to be an effective treatment modality for multiple cancer histologies [1]. Small molecules (SMs) and bioactive macrocyclic peptidomimetics and have been reported in the patent literature as PD-L1 binders able to block PD-1/PD-L1 interaction and immune checkpoint functions restoring T cells activity [2]. It has been demonstrated that SMs can induce a compound-dependent dimerization and subsequent internalization of PD-L1, effectively depleting the ligand from the cell membrane, avoiding immune response [3]. In this work, we set up a combined computational and biophysical protocol to shed light on the molecular recognition among PD-L1 inhibitors. In particular, we performed docking studies and molecular dynamic simulations to unlock the molecular basis of such interactions. Our study allows defining a preliminary pharmacophore model for PD-L1 ligands highlighting the essential residues for specific interactions. As a further characterization, we evaluated the binding affinity (Kd) trough microscale thermophoresis assays by biophysical assay such as Micro Scale Thermophoresis (MST). We have demonstrated that combining in silico and biophysical methods can be a powerful strategy for the investigation of binding interactions between PD-L1 and inhibitors, where data obtained provides clues for the rational design of new small molecules as functional peptidomimetics.

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TARGETING THE VIRAL ENVELOPE: SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL BROAD-SPECTRUM ANTIVIRALS

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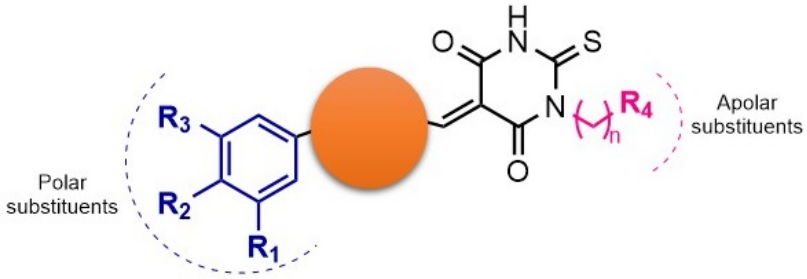
Some of the most infectious emerging and re-emerging viruses are endowed with a lipid membrane called envelope. Despite the viral envelope derives from the lipid membrane of the host cell, several differences can be highlighted between them, such as the lack of biogenic and reparative pathways that makes these viruses vulnerable to envelope's injury.

In our previous papers, [1,2] we reported some rhodanine and aminothiazolone derivatives endowed with submicromolar activities against HIV-1 infected cells. The compounds were found to be only moderately active on HIV-1 integrase and HIV-1 gp120, but their submicromolar activity in vitro on HIV-1 replication and time of addition experiment suggested a diverse mechanism of action.

Herein we reported novel analogues of a series of thiobarbituric acid derivatives [3], which displayed a broad-spectrum antiviral activity against different enveloped viruses (i.e. HSV-1, HCMV, RSV, ZIKV, INFLUENZA A, VSV) and resulted to be completely inactive against non-enveloped ones (i.e. Ad5, HPV and HRoV), suggesting that their mechanism of action could involve the viral envelope, affecting the dynamics of viral fusion and altering the fluidity and integrity of the lipid bilayer.



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RATIONALLY DESIGNED CASHEW NUT-SHELL LIQUID (CNSL)-TACRINE HYBRIDS AS SUSTAINABLE TREATMENT FOR ALZHEIMER'S DISEASE

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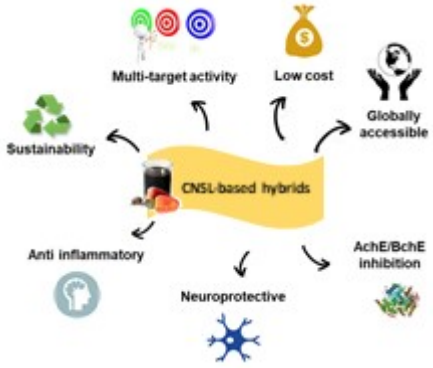
Alzheimer's disease (AD) is the most common form of dementia and a global health problem. A big increase in longevity and the lack of treatments are the reasons for an ever-increasing number of people affected by the disease AD worldwide, particularly in developing countries. Nowadays, the cost of the drugs in use is too high and is important to provide affordable and globally accessible medications. With these concepts in mind, we already explored the potentiality of cashew nut-shell liquid (CNSL), zero-cost, inedible by-product material, as a starting point for a sustainable drug discovery aimed to design multi-target hybrids. CNSL components are made by long-chain phenolic compounds, such as anacardic acid, cardanol, and cardol with innate multi-target mechanisms of action including anti-inflammatory and antioxidant activity, [1] which are beneficial for the treatment of neuroinflammatory-based diseases like AD. Our previous works demonstrate that the use of this inexpensive food waste materials could be successfully applied for the development of accessible and sustainable drug candidates for the treatment of AD, suggesting that the approach to obtain potential anti- AD drugs from CNSL is worth further pursuit and development. [2,3] Thus, we decided to design a series of CNSL-based hybrid compounds, building on the strategy that the combinations of two different fragments into a unique covalently linked hybrid compound, can convey synergy and increase potency. [4,5] Particularly, we combined the chemical features of CNSL derivatives with those of tacrine, renowned AchE inhibitor. The series of hybrids we synthesized was preliminary screened to test their hepato- and neurotoxicity. To assess the multi-target mechanism of action, neuroprotective and neuroinflammatory assays were performed together with inhibitory activity on cholinesterases that gave remarkable IC_{50} values, spanning from a wide range from nanomolar to picomolar. We envisage that such hybrids, obtained from renewable and inexpensive material, might be promising bio-based sustainable hits for anti-AD drug discovery.



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GLYCOCONJUGATES OF TRITERPENOIDS AND N-ACETYL-D-GALACTOSAMINE - PROMISING AGENTS AGAINST LIVER DISEASES

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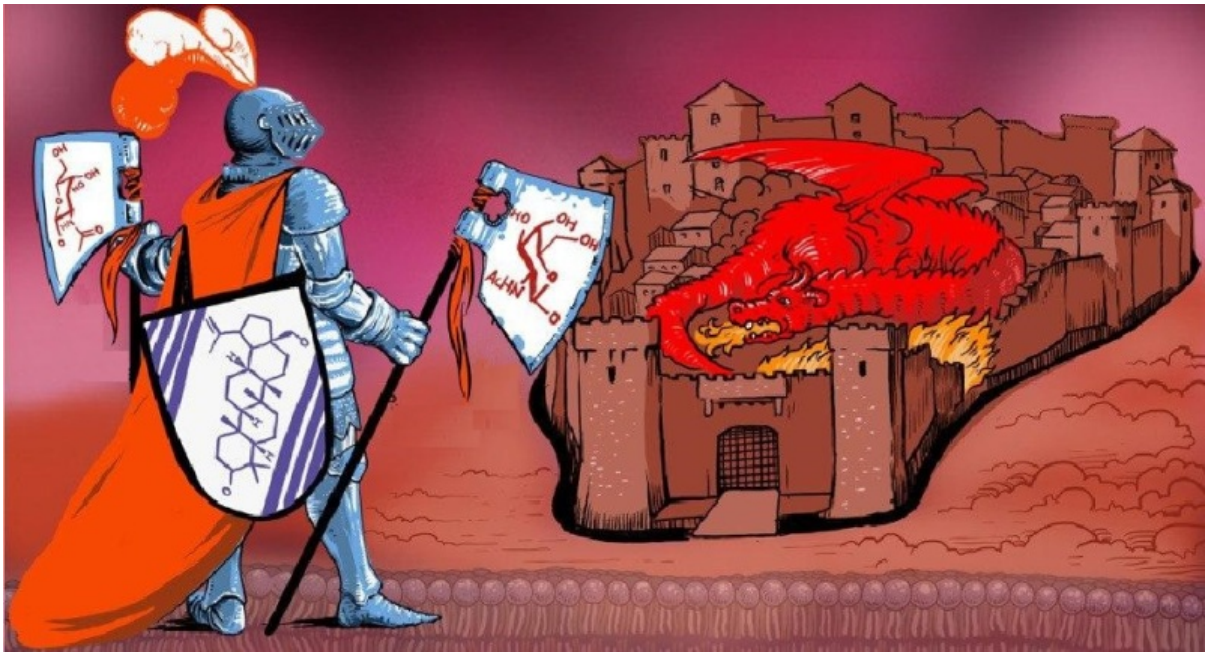
The development of new, effective drugs for the treatment of liver diseases is an important task in modern society. Diseases such as hepatocellular carcinoma, hepatitis B and C are directly associated in the human body with the liver and hepatocytes, which represent the major group of cells in the liver. The above diseases affect a large part of the human population and, in many cases, lead to serious consequences and lethal effects. For example, the viruses that cause hepatitis B and C may lead to chronic hepatitis, which can cause cirrhosis and liver cancer. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and accounts for 90% of all liver cancers (782,000 deaths were associated with HCC in the year 2018). To date, there is no universal pill that guarantees a complete cure in each case.

Pentacyclic triterpenoids are a class of natural compounds widely represented in plant sources. It is known, that triterpenoids are characterized by diverse biological activity, especially antiviral and antitumor. However, most terpenoid structures are characterized by common drawbacks – low solubility and bioavailability, weak pharmacological effects at low concentrations.

At present work, a new series of triterpenoid based glycoconjugates were synthesized and characterized. Initially, the primary alkyne derivatives of betulinic, oleanolic, ursolic, and glycyrrhetinic acids were obtained using different esterification reactions. Subsequent CuAAC-conjugation with azido-derivatives of N-acetyl-D-galactosamine (GalNAc) led to selective formation of new triterpenoid based glycoconjugates. Generally, 24 new compounds were synthesized during the study. Residues of GalNAc are known to provide selective binding of conjugates with ASGP-receptor on the surface of hepatocytes and thus to facilitate targeted delivery of drugs to the liver.



All compounds were screened for cytotoxicity in vitro against the HepG2, Huh7, and several control cell lines. SPR-spectroscopy studies of Kd values for conjugate-ASGPR complex showed the binding affinity, comparable to the conventional branched ASGPR-ligands. Chemical and enzymatic stability studies showed the ability of presented compounds to degrade under physiological conditions. Results of subsequent biological evaluation of the obtained glycoconjugates will be published separately during the current investigations.

This work was supported by the Russian Foundation for Basic Research (grant № 18-33-20106) and by Grant of the Republic of Bashkortostan for young scientists in 2020.

THOROUGH UNMASKING OF PROMISCUITY IN MEDICINAL CHEMISTRY

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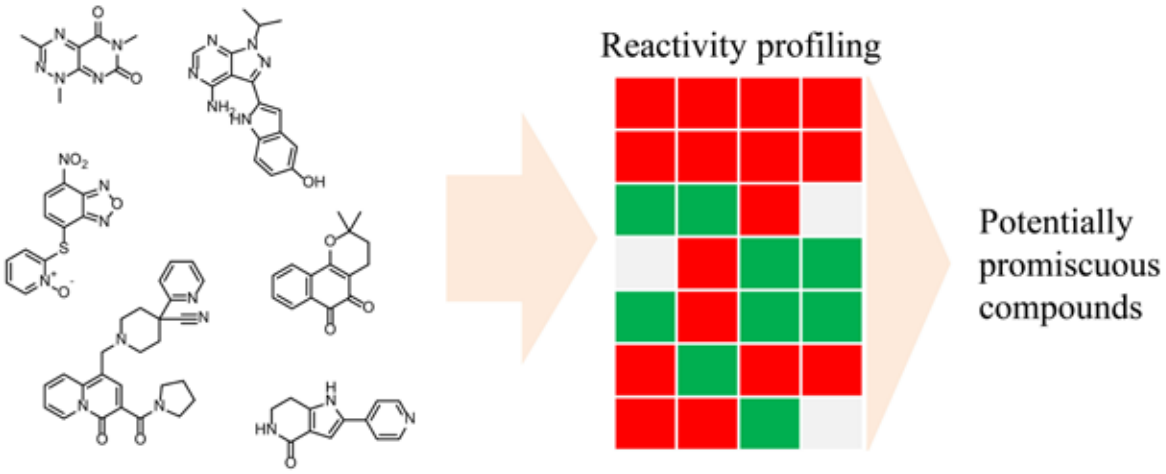
The identification of false positive hits is a crucial step in the early stages of drug discovery to avoid wasteful use of resources. Moreover, poor tool compounds in biological studies can generate false information about biochemical processes. The underlying promiscuous mechanisms of interaction with biological targets include aggregation, reactivity with protein residues, redox activity, interference of the assay system (PAINS), poor stability or solubility issues.

There are well established methods for the detection of aggregators, and many interfering compounds can be filtered before screening, e.g., using PAINS filters.[1] We present guidelines for the detection of less obvious promiscuous mechanisms, namely redox activity and thiol reactivity. After a thorough literature review, selected assays were optimized and tested with 10 chemically distinct positive control compounds. Then we have further expanded our set of compounds that can be used as positive controls. Assay setups are quick and can be performed at low cost. They are orthogonal and capable of detecting multiple interference mechanisms, i.e. the horseradish peroxidase–phenol red assay detects H_2O_2 generation; the probe H_2DCFDA is sensitive to ROS; resazurin reacts with free radicals; and the Ellman's reagent detects covalent reactivity with thioles. Assay conditions have been optimized to improve robustness and sensitivity.

A library of 99 chemical probes and bioactive compounds was used to demonstrate the capabilities of our assay cascade. We have identified new types of compounds that were not previously known to be redox active. Even well prepared, filtered and manually curated compound libraries can still contain nuisance compounds that can only be identified experimentally.



In summary, we have selected and optimized four different high-throughput assays, each of which has its own mechanism for the detection of redox active or thiol reactive compounds. The assays provide the utility of rapid identification of promiscuous compounds among screening hits or chemical probes. It is important to raise awareness of the potential for redox activity, as simple assays like these can be performed on a large scale and at an early stage of drug discovery.

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CONSENSUS DOCKING STUDIES FOR THE DEVELOPMENT OF NEW COLCHICINE BINDING SITE INHIBITORS WITH POTENTIAL ACTIVITY TUBULIN POLYMERIZATION DESTABILIZERS

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The concept of microtubule modulation, which leads to cell cycle blocking, represent an important strategy for anti-cancer therapies, currently used in chemotherapy treatments. This approach has been widely studied especially after the discovery of modulators able to interact with tubulin in the so-called colchicine binding site, which is capable of harbouring compounds with low molecular weight and volume - when compared to traditional inhibitors used in chemotherapy treatments, such as those derived from vinca alkaloids -, and also because they reveal interesting results concerning the diminishment of chemotherapy resistance in cancer, called multiple drug resistance (MDR)[1,2]. Given the importance in the development of new compounds that can be used in anti-cancer therapies, we have developed a virtual screening study aiming to achieve tubulin modulators that act on the colchicine binding site, using ligand- and structure-based techniques. The structure used in this study was the PDB ID: 4O2B[3] and its crystallographic ligand, the colchicine. To ensure the effectiveness of this study, virtual screening techniques were validated by recovery of known active ligands from a dataset containing decoys generated by DUD-E[4] platform, for both similarity and docking studies, and also by performing redocking for docking studies. The six databases used in this study - Maybridge subdivision Screening Collection, ZINC subdivisions natural products and Drug database, Chembridge subdivisions DIVERSet™-EXPEXPRESS-Pick™ Collection (DIVERSet™-EXP), DIVERSet CORE Library (DIVERSet™-CL) and BindingDB drug database - were prepared using the OMEGA software which was used to generate up to 300 conformers per molecule. Then, screening was carried out sequentially by applying shape similarity using the ROCS software, electrostatic similarity using the EON software, ADME/Tox predictions using the QikProp and DEREK software, respectively, and docking simulations. The docking simulations were performed using the Glide and GOLD software so that the final selection of compounds could be obtained through consensus analyses of corresponding interaction modes of survival compounds, associated with a thorough visual inspection. Thus, we obtained 10 promising hits as candidates for tubulin modulation aiming the interaction with the colchicine binding site.

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DOUBLE-ATTACK STRATEGY TO TACKLE NEUROINFLAMMATION WITH HYBRIDS TARGETING NRF2 AND MAO-B: A PRELIMINARY STUDY

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Neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, are strictly correlated to neuronal death and loss of synaptic function, leading to the distinctive progressive motor and cognitive decline. Neuroinflammation plays a pivotal role in triggering the neurodegenerative pathological cascades through impairment of pro/antinflammatory cytokine production with derived onset of proteinopathies and oxidative stress. Neuroinflammatory conditions, as a consequence of glial cells activation, involve alterations in enzyme functions/expressions (e.g. monoamine oxidase, MAO) and genes transcription (e.g. nuclear factor erythroid 2-related factor 2, Nrf2-Keap1 pathway) physiologically responsible for oxidative balance and cytokines release. MAOs degrading neurotransmitters produce ROS. Isoform MAO-B is mainly centrally expressed, its expression increases with age and its biological activities are regulated by astrocytes in physio/pathological conditions: all these outputs make MAO-B an interesting target for studying age-related neuroinflammation.[1] On the other hand, Nrf2 is a transcription factor, physiologically trapped in the cytosol by Keap1, key regulator of cellular redox homeostasis, whose hyperactivation has shown important therapeutic effect in *in vivo* neuroinflammatory models.[2] Taken these premises, we envisioned to merge in a single chemical entity pharmacophores able to inhibit MAO-B and activate Nrf2 downstream signalling and we biologically evaluated how this double-attack strategy can modulate or interfere with neuroinflammatory cascades. Particularly, we selected pro/electrophilic moieties of hydroxycinnamic derivatives as efficient Nrf2 activators and pioglitazone, a peroxisome proliferator-activated receptor gamma (PPARγ) agonist, which has been recently repositioned as strong, selective and reversible MAO-B inhibitor.[3, 4]

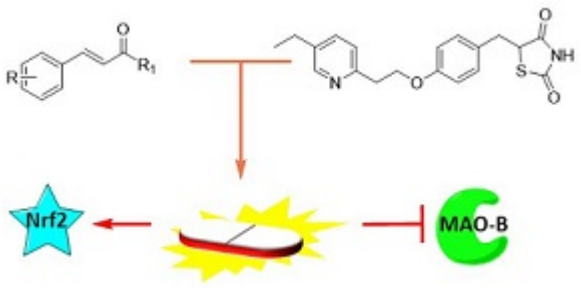

Figure. Molecular hybridization approach based on hydroxycinnamic-pioglitazone scaffolds.

Resulting hybrids were preliminary evaluated for MAO-B inhibitory activity and selectivity and for the ability to induce Nrf2-driven antioxidant response, proving to show promising and balanced biological profile for the targets of interest.

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INVESTIGATION OF THE FIRST MN(II)-BASED CONTRAST AGENT CANDIDATE FOR SENSING Zn(II) ION IN VIVO

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The MRI (Magnetic Resonance Imaging) technique provides the opportunity to investigate the biochemical processes on molecular level. The molecular imaging - instead of anatomical imaging - requires special molecular imaging probes which can give response for the change of the investigated parameter. The benefit of these methods would be the deeper understanding of biochemical abnormalities for earlier diagnosis. [1]

Many tissues can release Zn(II) ions to response external stimuli. Here we report that the secretion of Zn(II) ions from healthy prostate tissues stimulated by glucose in fasted mice can be monitored by MRI using our Mn(II) based complex. The values of the equlibrium investigations show the complex formation is practically 100% at pH 7.4. The inertness of the Mn(II) complex was studied by transmetallation reaction using ^{1}H relaxometry that shows the half-life of the complex is high enough for *in vivo* application. [2]

The relaxivity values of the [Mn(PC2A-DPA)] and the [Mn(PC2A-DPA)Zn]$^{2+}$ complex were examined using ^{1}H relaxometry in different magnetic field strengths, temperatures and various matrix (HSA, Seronorm). The efficacy of the [Mn(PC2A-DPA)] complex to detect the Zn(II) ion was studied by *in vitro* (phantom) and *in vivo* (using healthy BALBC/c mice) MRI at 1.5 and 3 T magnetic field strengths. The results of *in vivo* examinations shows (Figure 1.) that the contrast enhancement is around 20% higher than the native at the absence of D-glucose, but after i.p. injection of D-glucose the increase of the contrast is significant (approx. 60% higher than native).

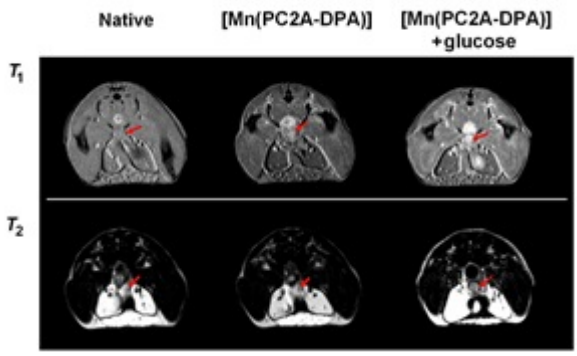

Figure 1. Representative transaxial T_1- (upper row) and T_2-weighted (lower row) MR images of the prostate of healthy male mice at 3 T magnetic field. From left to right: pre-injection (native) and post-injection of [Mn(PC2A-DPA)] without and with the i.p. co-injection of D-glucose. The red arrows indicate the prostate.

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SYNTHESIS AND ANTI-MITOTIC ACTIVITY OF 2,4- OR 2,6-DISUBSTITUTED- AND 2,4,6-TRISUBSTITUTED-2H-PYRAZOLO[4,3-c]PYRIDINES

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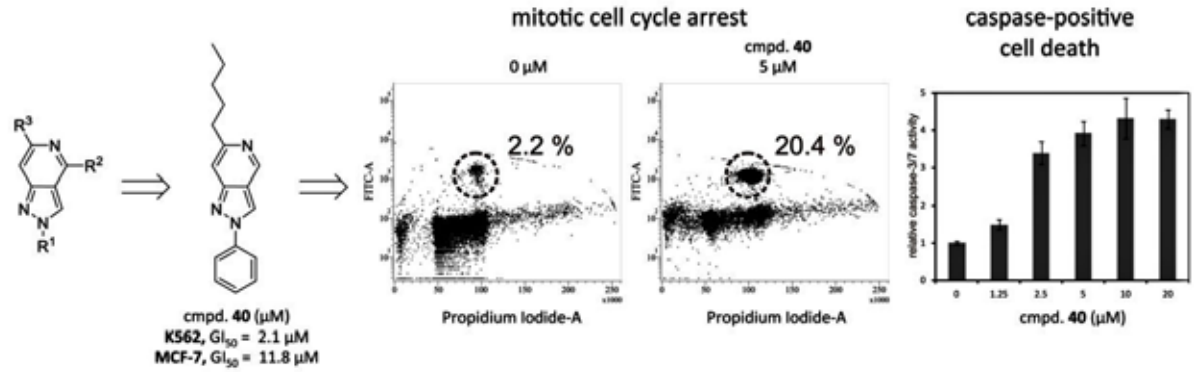
Organic compounds possessing pyrazole nuclei are of interest to medicinal chemists and materials scientists, thus their synthesis is a worthwhile task. This is especially true for fused ring systems containing pyrazole unit due to biological activities associated with them. Noteworthy, annulated pyrazoles constitute the core of several well-known drugs, including Sildenafil, Zaleplon and Allopurinol.
Herein we present synthesis and biological activity of novel 2*H*-pyrazolo[4,3-*c*]pyridine derivatives easily accessible from various pyrazolidin-3-ones. The newly synthesized compounds were screened for their anticancer activity and some of the derivatives demonstrated promising micromolar inhibition against K-562 and MCF-7 cell lines. Moreover, the most potent compound **40** caused mitotic cell cycle arrest leading to apoptosis.



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SYNTHESIS AND EVALUATION OF ANTHELMINTIC ACTIVITY OF NOVEL BENZOPYRANO[2,3-c]PYRAZOL-4(2H)-ONES

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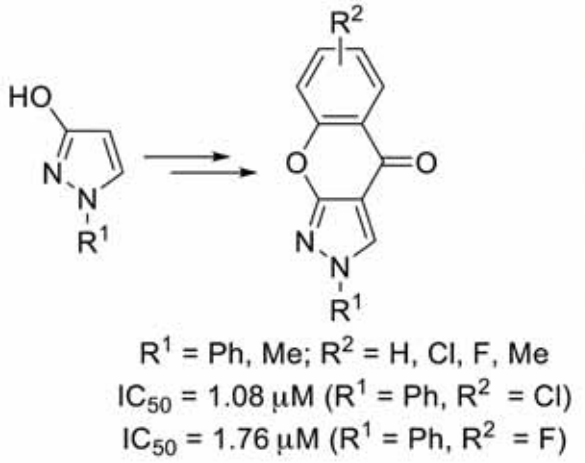
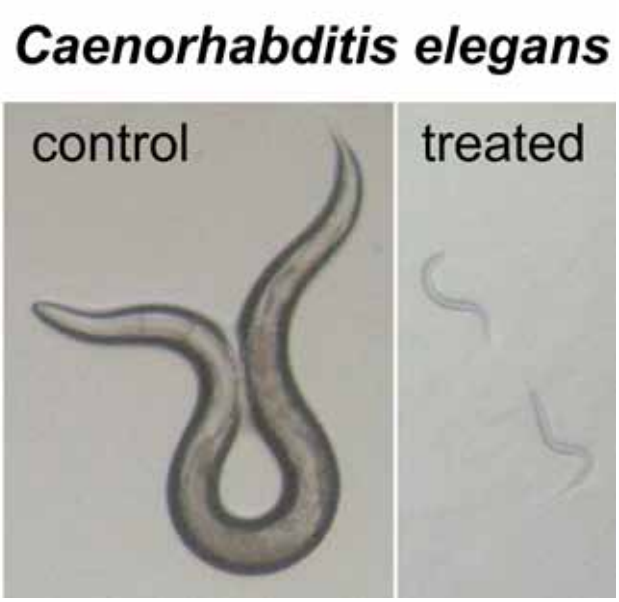
Condensed pyrazole ring possessing heterocycles attract significant interest due to their vast biological and pharmacological activities.

In this work an efficient three-step synthesis of benzopyrano[2,3-*c*]pyrazol-4(2*H*)-one derivatives, varying by the substituents at the 2-, 6-, 7-, and 8-positions, was developed sequentially employing *O*-acylation, Fries rearrangement and potassium carbonate induced cyclization. The anthelmintic properties of the obtained compounds were investigated *in vivo* in a model nematode, *Caenorhabditis elegans*. Five compounds, namely 2-phenyl[1]benzopyrano[2,3-*c*]pyrazol-4(2*H*)-one and its 7-fluoro, 7-chloro-, 7-bromo- and 8-fluoro-analogues, altered the development of *C. elegans*. The compounds strongly inhibited the development of the worms, with the majority of larvae never progressing past the L1 stage. [1].





Acknowledgements:

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NOVEL ADAMANTANE-BASED TDP1 INHIBITORS HAVING MONOTERPENOID AND HETEROCYCLIC FRAGMENTS

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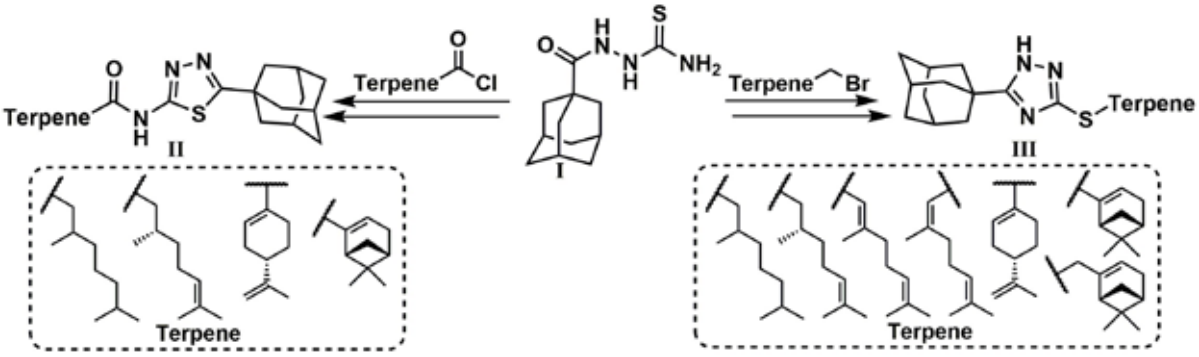
Camptothecin derivatives, such as topotecan and irinotecan, are among the most common drugs being used in the treatment of cancer [1]. The mechanism of action of these drugs is associated with inhibiting topoisomerase 1 (Top1), an enzyme, that plays an important role in cell division processes. However, there are a number of problems related to this type of therapy, in particular an ability of DNA repair system to remove damage caused by antitumor drugs. Tyrosyl-DNA phosphodiesterase 1 (Tdp1) is considered to play a key role in the repair of DNA lesions, thus preventing cancer cell death. Therefore, developing Tdp1 inhibitors is of great interest in modern medicinal chemistry as they could act synergistically with Top1 inhibitors in cancer combination therapy.

Adamantane derivatives as well as substances containing 1,3,4-thiadiazole and 1,2,4-triazole fragments have found wide application in medicinal chemistry [2,3]. On the other hand, structural modification of natural metabolites is one of the most fruitful approaches for the development of potential drugs. Monoterpenoids and their derivatives exhibit a number of biological activities, such as antibacterial, anti-inflammatory, antiviral, anticancer etc.

In an attempt to combine these structural blocks, namely adamantane, 1,2,4-triazole/1,3,4-thiadiazole and monoterpenoid moieties in one molecule, we have synthesized compound **I** that has been transformed into the corresponding 1,3,4-thiadiazole **II** and 1,2,4-triazole **III** derivatives under alkaline and acidic conditions respectively, followed by the modification of heterocyclic compounds with monoterpenoid residues having acyclic, monocyclic or bicyclic structures.



The compounds obtained were tested for their Tdp1 inhibitory properties. All the compounds were found to exhibit inhibitory activity at submicromolar and micromolar concentrations. Among thiadiazoles **II**, the amides of 3,7-dimethyloctanoic or (-)-myrtenic acids demonstrated the most pronounced activity, with IC_{50} being 0.35±0.05 and 0.45±0.09 μM, respectively. As for triazoles **III**, the highest IC_{50} value was found for compounds containing (-)-nopol (0.57±0.14 μM) and 3,7-dimethyloctanol (0.54±0.09 μM) moieties. (-)-Myrtenyl derivative of 1,2,4-triazole **III** proved to be the least active with an inhibitory effect on Tdp1 at a concentration of 7.50±1.80 μM, whereas thiadiazole **II** compound having the analogous substituent showed the best inhibitory properties. It is worth noting that 1,3,4-thiadiazoles **II** tended to demonstrate higher activity compared to 1,2,4-triazoles **III**.

This work is supported by the Russian Science Foundation under grant 19-13-00040.

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INVESTIGATION OF [Al18F]2-RADIOFLUORINATION IN DIFFERENT LABELLING CONDITIONS

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Positron emission tomography is a non-invasive molecular imaging technique, which provides functional imaging of human body. In the clinical practice, the most commonly used positron emitting isotope is the ^{18}F radioisotope, which has ideal properties ($t_{1/2}$ = 110 min, I= 97% $E(\beta^+)_{max}$ = 635 keV) for PET imaging. The conventional radiofluorination is based on nucleophilic substitution reaction requires generally anhydrous and hard conditions. However, McBride *et al.* developed a new radiofluorination technique, which is conducted by aqueous conditions, hence this method makes easier the ^{18}F labelling of peptides and macromolecules. This new radiofluorination process based on the complexation of 1,4,7-triazaciklononane derivatives with $[Al^{18}F]^{2-}$.[1]

The aim of our research were the preparation of two precursors containing 1,4,7-triaza-cyclononane-1,4-diacetic acid (NODA) and investigation of the effect of the different labelling parameters (reaction time, temperature, the presence of ethanol, the amount of precursor and $AlCl_3$ solution) for their radiofluorination with $[Al^{18}F]^{2-}$. The synthesis of the known $Al^{18}F$-NODA-NI for tumour hypoxia imaging, and the novel $Al^{18}F$-NODA-cRGDfK dimer for detection of $\alpha_V\beta_3$ positive tumours with PET were achieved. The logP value and the stability test of the synthetized radiotracers were determined. In addition to the biological investigation of $Al^{18}F$-NODA-NI radiopharmaceutical was accomplished.

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IN VITRO BIOLOGICAL SCREENING OF DHA BASED BENZODIAZEPINE AND CHELATES

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There is a growing interest on DHA derivatives compounds in recent years, due to an establish antibacterial, antifungal, anti-inflammatory and anticancer activities, several researches were performed on Benzodiazepine and metal complexes derivatives. [1,6]

Efficient and easy access to different classes family using dehydroacetic acid DHA as starting materials is reported, the obtained compounds were fully characterized by UV-Vis and IR spectroscopy in addition to X ray diffraction on monocristal, several *in vitro* biological tests were also performed on this new series of compounds to explore their therapeutically potential in order to continue further investigations and exploring them as new target drugs. In this case, antioxidant activities, enzymatic activity and antimicrobial activity against several bacterial and fungal referenced strains, exhibit interesting results which will be developed in this work .

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EVOLUTION OF JASMONATE-SCAFFOLDED PPAR-GAMMA AGONISTS: A MOLECULAR MODELLING STUDY

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Jasmonates are plant hormones involved in damage responses and developmental processes. They are chemical analogs of prostaglandins and have been proved to cross-talk with animal and human inflammatory processes exerting strong anti-inflammatory effect. This effect is mediated by PPARγ receptor activation. This action was assumed to be due chemical similarity between jasmonates and 15d-PGJ_2, anti-inflammatory prostaglandin covalently activating PPARγ. But the carbon chains in jasmonate structure are shorter than in prostaglandins. So, in our previous work, we assumed that jasmonates cannot bind to PPARγ like 15d-PGJ_2 and bind like non-steroidal anti-inflammatory drugs (NSAIDs) [1]. So, during last years, significant advances were made im biochemical and bioinformatic study of jasmonates. Firstly, it has been revealed that enone functionality in a jasmonate molecule is required for PPARγ activation [2] that proves covalent linking of jasmonate to Cys285 residue like in the case of 15d-PGJ_2. Secondly, some jasmonate-scaffolded PPARγ agonists were synthesized and proven to be potent [2, 3]. A molecular modelling studies showed that these agonists can bind the receptor like 15d-PGJ_2. These findings contradicted our study. Furthermore, in these articles no molecular-modelling evidence of covalent linking was provided, like it was done in the paper [4] for plakilactones: hydrogen bond because potential Michael-reacting groups is an evidence of covalent bonding in real circumstances. So, the aim of our work was to perform detailed molecular modelling study to refine the mechanism of PPARγ activation by jasmonates, including novel compounds.

We used fully flexible docking with ROSIE Ligand Docking protocol [5] and AutoDock Vina [6]. Using BIOVIA Discovery Studio Visualizer program, we revealed that novel jasmonates (i. e. compound 6a1 and compound 5f) form a hydrogen bond between a carbonyl group and Cys285, which is an evidence of covalent attachment similar to 15d-PGJ_2 bindng. The cyclopentenone rings of jasmonates bind like those in 15d-PGJ_2. A novelty of tail groups of compound 6a1 and compound 5 is to bind very similar to 15d-PGJ2 acyclic tail. The head group of compound 6a1, like in methyl jasmonate and many other compounds, is too short to form all hydrogen bonds like 15d-PGJ_2 and binds only with the nearest residues of the hydrophilic subpocket: Tyr237 and His449. Compound 5f, the most novel jasmonate, has long polar head that better mimics hydrogen bonds of 15dPGJ_2, including bond with Tyr437, and binds like 15d-PGJ_2 carboxyl group.

Our molecular modelling results show that the potency of jasmonate agonists of PPARγ is linked with the binding similarity to 15d-PGJ_2 binding. The more similar jasmonate binds, the more potent it is. We traced the structure evolution of novel jasmonates and assumed, that their development wend the way of coming closer to 15d-PGJ_2 structure. We concluded, that, in an extreme case, it will lead to prostaglandine scaffold. Our practical recommendation is to use prostaglandins, but not jasmonates, as a lead compounds.

However, we concluded that jasmonates bind to PPARγ like prostaglandins, but natural jasmonates used in earlier works are not fully-featured prostaglandin mimic. This action is an interest biological example of cross-kingdom oxylipin signaling.

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MICELLAR BRØNSTED ACID-MEDIATED SYNTHESIS OF DNA-TAGGED HETEROCYCLES

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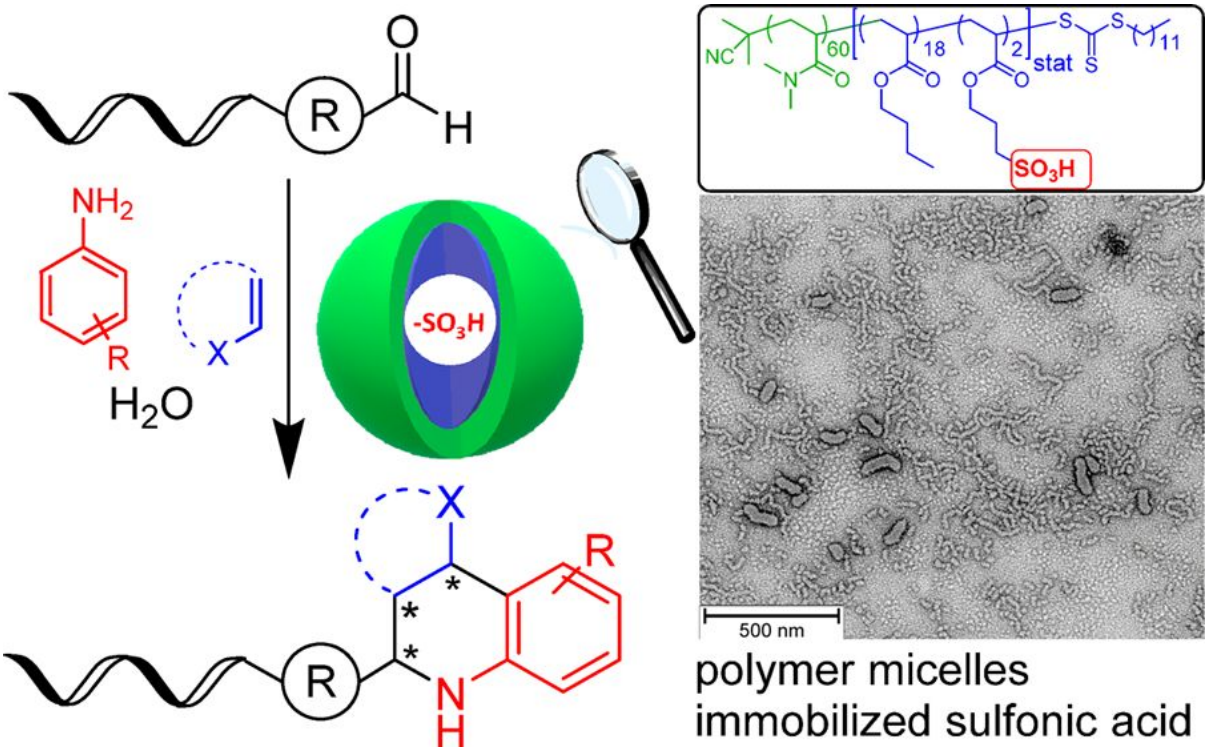
The translation of established molecular biology methods such as genetic coding, selection, and DNA sequencing to combinatorial organic chemistry and substance identification has made extremely large compound collections, so-called DNA-encoded libraries, accessible for drug screening. However, the reactivity of DNA limits the choice of chemical methods for the synthesis of encoded libraries. For example, strongly acidic reaction conditions must be avoided, as they damage the DNA by depurination, i.e. the cleavage of the purine bases from the oligomer. The application of micellar catalysis is promising for encoded chemistry. Amphiphilic block copolymers, consisting of a hydrophilic polyacrylamide portion and a hydrophobic poly(butyl acrylate) portion, which is statistically functionalized with sulfonic acid residues, accumulate in water to form micellar aggregates. These acidic nanoreactors enabled the reaction of DNA-conjugated aldehydes to different substituted tetrahydroquinolines and aminoimidazopyridines by Povarov or Groebke-Blackburn-Bienaymé reactions and the cleavage of Boc protective groups from amines.[1] Future research involves investigations in the use of micellar catalysis for screening library synthesis.



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IN SILICO RATIONAL DRUG DESIGN AND MODELLING STUDIES OF NOVEL 5-HT2A RECEPTOR ANTAGONISTS

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Alterations in a serotonergic system are mainly associated with neurological diseases and neuropsychiatric disorders.[1] Therefore, antagonists of serotonin 5-HT_{2A} receptors (5-HT_{2A}R) appear to be very effective in treatment of depression, insomnia, schizophrenia, anxiety, and Parkinson's disease.[2] The development of novel 5-HT_{2A}R antagonists, through analysis of 3D-structure of the pharmacophore as well as binding kinetics of known compounds, appears to be an interesting topic in various areas. In this context, we have combined three-dimensional quantitative structure-activity relationship (3D-QSAR) modelling with molecular docking and molecular dynamic (MD) simulation in order to find out structural features crucial for antagonistic activity. Data set was composed of 75 structurally diverse antagonists, divided into three different clusters, based on their chemical structures: clozapine, ziprasidone, and ChEMBL240876 derivatives. Firstly, each cluster representative in complex with 5-HT_{2A} receptor was submitted to 50ns long MD simulation to obtain their inactive, antagonist-bound conformations. Subsequently, obtained conformations were used as templates for docking studies in order to generate virtually bioactive conformations of all studied ligands, as well as to investigate their binding modes in the active site of the receptor. Selected conformers were further used for calculation of specific molecular descriptors and 3D-QSAR model building. Statistically significant variables were used for clarifying the most important structural features required for 5-HT_{2A} antagonistic activity. Good predictive power and stability of the created 3D-QSAR model was confirmed with different internal and external validation methods. Moreover, the results of 3D-QSAR, molecular docking and MD studies showed good concordance. To sum up, these results collectively enable us to produce innovative workflows for the rational drug design of novel compounds.

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SYNTHESIS AND PHOTODYNAMIC PROPERTIES OF PYRAZOLE-INDOLE HYBRIDS IN G361 CELL LINE

Gabrielė Varvuolytė (1), Lukáš Malina (2,3), Aurimas Bieliauskas (1), Barbora Hošíková (2), Helena Simerská (4), Hana Kolářová (2), Neringa Kleizienė (1), Vladimír Kryštof (3,4), Algirdas Šačkus (1,5), Asta Žukauskaitė (4)

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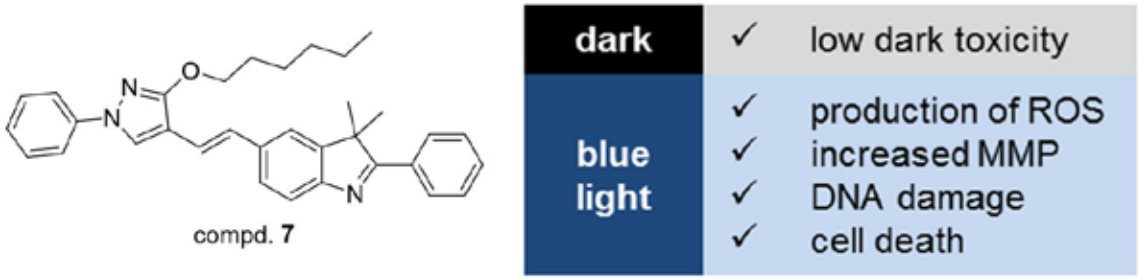
Photodynamic therapy (PDT) is a non-invasive treatment for various skin conditions and cancers that uses a light-sensitive medication (photosensitizer), light source, and oxygen to produce reactive oxygen species which can cause the destruction of abnormal cells [1,2]. Compounds with elongated π-conjugated systems often possess photodynamic properties, however, to be suitable for PDT they also need to meet a number of requirements, such as low dark toxicity, strong photocytotoxicity, accumulation in target cells, etc. Therefore, the synthesis of new types of photosensitizers remains relevant in current organic chemistry.

New pyrazole-indole hybrids were synthesized from 3-(hexyloxy)-1-phenyl-1*H*-pyrazole-4-carbaldehyde and 5-iodo-3,3-dimethyl-2-phenyl-3*H*-indole *via* olefination, Vilsmeier-Haack formylation and Pd-catalysed cross coupling reactions. Their dark toxicity was evaluated in G361 (malignant melanoma) cell line; the compounds displayed virtually no cytotoxicity unless irradiated with blue light (414 nm). Notably, compound **7** displayed EC_{50} values of 3.08, 0.26 and 0.05 μM in cells irradiated with 1, 10 and 50 J/cm^2 light dose, respectively. It increased levels of reactive oxygen species in treated cells and decreased JC-1 polymer aggregation within the mitochondria, suggesting mitochondrial membrane depolarization. Moreover, extensive DNA fragmentation was detected using comet assay as well as from increased histone H2A.X phosphorylation at Ser-139 and cleavage of PARP. We thus demonstrated that pyrazole-indole hybrids may serve as an interesting source of photosensitizing compounds with anticancer activity [3].



Acknowledgements

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DEVELOPMENT OF SMALL MOLECULE GPVI MODULATORS FOR THE TREATMENT OF THROMBOSIS

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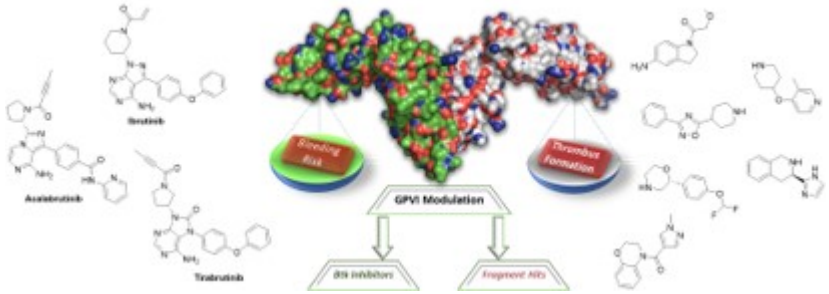
The formation of a blood clot (thrombus) is essential after injury to prevent excessive blood loss (haemorrhaging) and hypotension. In a disease state however, clots can also obstruct blood vessels, occluding the lumen and reducing oxygen delivery to tissues, which is known as 'thrombosis' This can result in myocardial infarction, stroke, deep vein thrombosis and pulmonary embolism. Worryingly, these diseases are responsible for 1 in 4 deaths worldwide,[1] and current epidemiological evidence estimates that 530 people in the UK will have a heart attack every day, and 190 of those people will die.[2] The existing antiplatelets such as aspirin have life-threatening side effects, including gastrointestinal and intracranial haemorrhaging.[3] Hence, an effective and safe treatment for thrombosis continues to be an area of unmet clinical need.

In the past few years the GPVI (glycoprotein six) receptor has emerged as a potential antiplatelet target. There are numerous preclinical models where genetic or peptide-induced GPVI deficient mice were determined to be protected from arterial or pulmonary thrombosis, as well as myocardial ischemia–reperfusion injury and ischemic stroke.[4] Furthermore, patients deficient in GPVI show only a mild bleeding phenotype (e.g. they often have epistaxis, petechiae) suggesting that a GPVI modulator could be a safer antiplatelet agent.[5] Unsurprisingly, a handful of small molecule GPVI antagonists have recently been reported, but these are mostly repurposed compounds with micromolar potency, and their binding to GPVI has not been sufficiently validated.[4]

The overall aim of this project was to develop a potent and selective small molecule GPVI antagonist *via* two concurrent approaches: (1) From the repurposing of existing Btk inhibitors, (2) From a novel fragment identification and enumeration strategy.

Btk is a protein expressed within B-cells and platelets. It has been reported that approved Btk inhibitors used for the treatment of blood cancers such as mantle cell lymphoma, were able to reduce collagen-induced platelet aggregation.[6] We have demonstrated that these compounds are also likely to be direct GPVI modulators, and SAR investigations are underway to optimise these compounds towards GPVI modulation. The lead compound from the Btk SAR investigation has also been used in shape-based virtual screening to discover a novel fragment hit.[7] The fragment has been validated in orthogonal functional assays, and direct binding to GPVI has been demonstrated using SPR. An SAR study on this fragment has been completed, and we are awaiting assay results prior to fragment elaboration.



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PEPTIDYL ACYLOXYMETHYL KETONES AS ACTIVITY-BASED PROBES FOR THE MAIN PROTEASE OF SARS-COV-2

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The global pandemic caused by SARS-CoV-2 calls for a fast development of antiviral drugs against this particular coronavirus. Chemical tools to facilitate inhibitor discovery as well as detection of target engagement by hit or lead compounds from high throughput screens are therefore in urgent need. We here report novel, selective activity-based probes that enable detection of the SARS-CoV-2 main protease. The probes are based on acyloxymethyl ketone reactive electrophiles combined with a peptide sequence including non-natural amino acids that targets the non-primed site of the main protease substrate binding cleft. They are the first activity-based probes for the main protease of coronaviruses and display target labeling within a human proteome without background. We expect that these reagents will be useful in the drug development pipeline, not only for the current SARS-CoV-2, but also for other coronaviruses.

STABILISERS OF TRANSTHYRETIN TETRAMERIC STRUCTURE: SYNTHESIS, BIOLOGICAL EVALUATION AND X-RAY CRYSTAL COMPLEXES

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Transthyretin (TTR) is a homotetrameric protein ß-sheet-rich which transports thyroxine (T_4) and retinol both in plasma and in cerebrospinal fluid. Recently, it has been reported that TTR also interacts with amyloid-β (Aβ) playing a protective role during Alzheimer's disease (AD) onset and progression [1]. *In vitro* experiment shows that TTR, in presence of Cu^{+2},binds Aβ in nM range forming a stable trimeric complex which can be responsible of Aβ scavenger action [2].

TTR is characterized by four identical monomers which interact each other through non-covalent bonds forming two pairs of dimers. Two dimers are assembled together by 2-fold symmetric axis making a tetramer which is crossed by two similar hydrophobic channels named T_4-binding sites (T_4-BS). Under physiological conditions, only a small amount of T_4 in the plasma is bound to TTR. Due to its intrinsic amyloidogenic potential, TTR can be responsible for certain amyloidotic diseases diseases. The stabilization of the TTR tetrameric state, by natural or synthetic small molecules, is a promising strategy to treat TTR amyloidosis[3][4]. The first-in-class drug discovered, inspired by this strategy and directed to the treatment of familial amyloid polyneuropathy, is Tafamidis meglumine (Vyndaqel ®). In this work we report the synthesis, the *in vitro* evaluation and the X-ray crystallographic analysis of a new series of biaryl compounds (Figure 1).

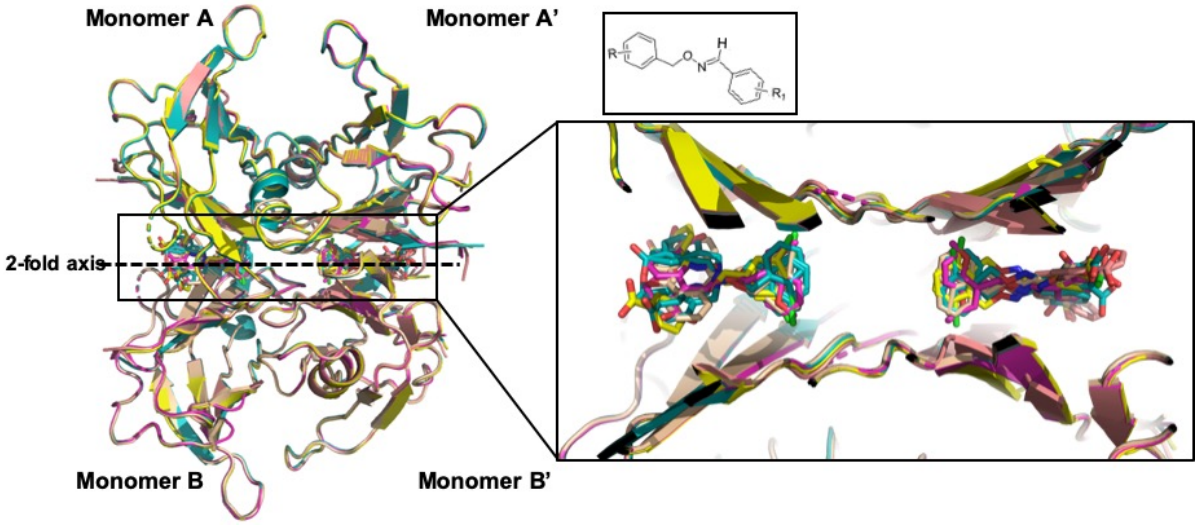

Figure 1. X-ray structure of TTR-biaryl ligands crystal complexes.

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ALKOXYAMINE ACTIVATED BY ENZYME AS A NEW WAY TO CURE CANCER

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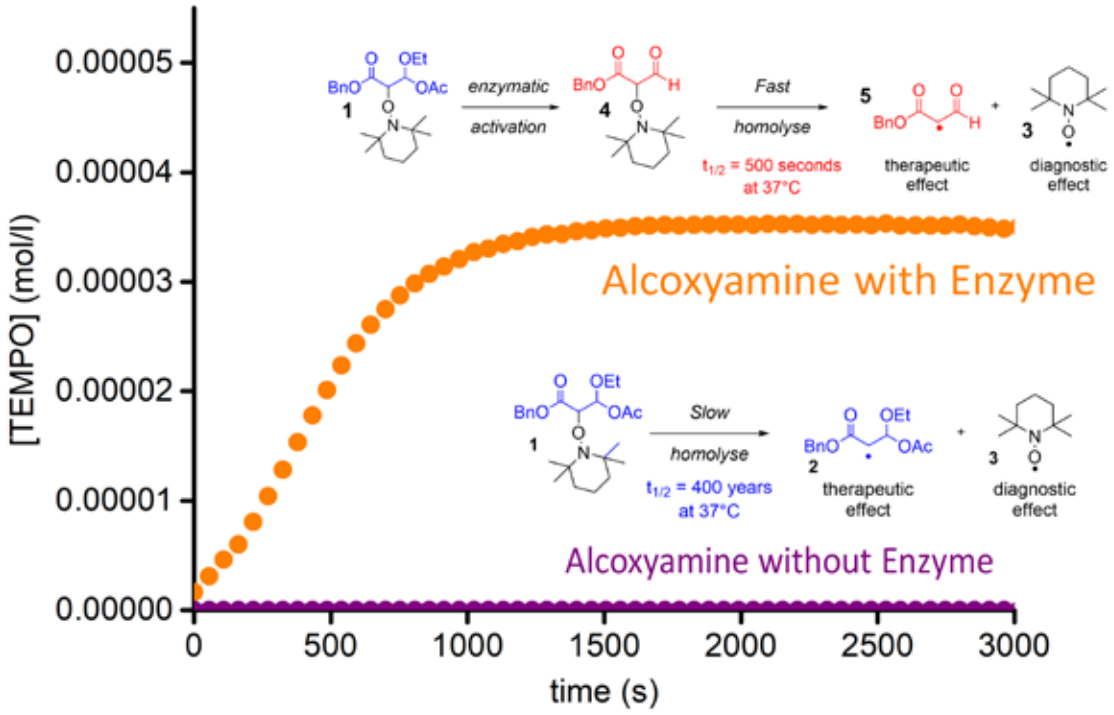
Alkoxyamines are well known since decades, because of their ability of generating free radicals by spontaneous homolyzes in an alkyl radical **2** or **5** and nitroxide **3** for example[1]. It has been shown that alkyl radicals are powerful cell killers[2]. In the same time it has been demonstrated that **3** is a good probe for imaging by Overhauser-enhanced Magnetic Resonance Imaging[3].



So with both effects the application of alkoxyamine **1** as theranostic agents model has been synthesised with the aim to be stable enough to be handled and quickly homolyzed upon enzymatic activation[4].

Next step deals with the preparation of an alkoxyamine carrying a non-symmetric acetal function with a specific peptide which releases a highly labile alkoxyamine upon selective enzymatic activity

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IMPROVED SYNTHESIS AND ISOLATION OF BEDAQUILINE

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Bedaquiline (BDQ) is the most critical pharmaceutical in the world for treating multidrug-resistant *M. tuberculosis*. Despite it being highly effective, BDQs asymmetric synthesis remains a challenge. Herein, the influence of chiral bases, namely bis(1-phenylethyl)amine, bisoxazoline, and sparteine on the diastereoselective lithiation reaction to obtain BDQ was investigated. The highest diastereoselective ratio (*dr*) emerged as 90:10 from the (+)-bis[*R*-1-phenylethyl] lithium amide. This is a significant improvement from the 50:50 *dr* achieved from the commercial synthesis. Thereafter, the desired (90:10 *RS, SR*) diastereomeric mixture was easily isolated via a gravity column and subject to chiral supercritical fluid chromatography (SFC) to access the desired enantiomer (*1R, 2S*)-BDQ. The advantages of this procedure are enhanced diastereoselection as well as a greener, faster way to achieve excellent enantioseparation (up to 1.0g scale).

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D-TOOLS: OPEN CHEMOINFORMATIC WEB SERVERS FOR DRUG DEVELOPMENT

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Online servers have an important role in drug discovery for target identification, lead discovery, and optimization [1]. The DIFACQUIM research group [2] has developed five free web services to analyze chemical diversity and chemical space, generate in silico libraries of peptides, and predict bioactivity profiles. The web services are collectively termed D-Tools: DIFACQUIM Tools for Chemoinformatics. In this contribution, we will discuss the development, implementation, and application of the five following web servers:

The Platform for Unified Molecular Analysis: PUMA: is a server for the diversity analysis of compound data sets [3]. Activity Landscape Plotter is aimed to perform structure-activity relationships using the concept of activity landscape modeling and identify activity cliffs. The server generates automatically structure-activity similarity (SAS) maps and Dual-Activity Difference (DAD) maps [4]. Consensus Diversity Plot is valuable to analyze compound data sets using different measures of diversity simultaneously; hence, it enables a "global" diversity analysis [5]. D-Peptide Builder is a server for the construction of peptide combinatorial libraries. The application also computes the molecular properties of the new combinatorial libraries, generates an online visual representation of the chemical space, and performs a diversity analysis [6]. Epigenetic Target Profiler is an online application to predict the bioactivity profile of user input structures with a panel of more than 30 epigenetic targets. All servers are freely accessible at https://www.difacquim.com/d-tools

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BINDING MODE PREDICTION OF NEWLY SYNTHESIZED SPIROCYCLOPROPYL OXINDOLES AS HIV-1 RT INHIBITORS

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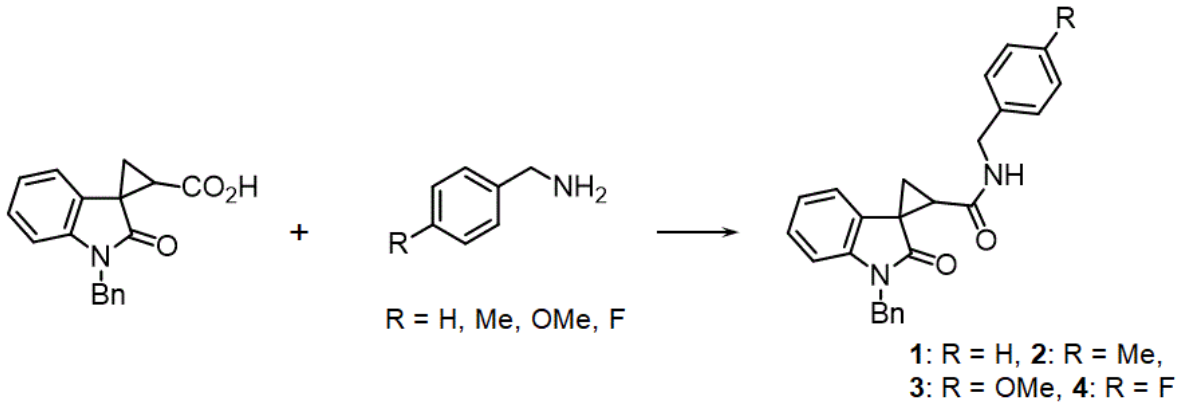
Abstract: In the case of human immunodeficiency virus type 1 (HIV-1) infection, reverse transcriptase (RT) enzyme is one of the most successful approaches for the treatment of HIV-1 infections. Among the HIV-1 RT inhibitors, non-nucleoside reverse transcriptase inhibitors (NNRTIs) has grown increasingly in the last two decades due to significantly better success rates in the development phase than for alternative treatments based antiretroviral therapy (ART).[1] The design of better NNRTIs is also motivated because reverse transcription is an extremely fast process allows the virus to develop viral progeny which can result in mutations associated with HIV-1 drug resistance, with the consequently reduced efficiency of many NNRTI drugs.[2] Here, we explore the binding pattern of newly synthesized spirocyclopropyl oxindoles **1**-**4** against the target HIV-1 RT enzyme using molecular docking approach. **Methods and Results:** The core structure of compounds **1**-**4** was selected considering their 'butterfly'-shaped structural pattern, that characterizes NNRTI drugs, which bind at the hydrophobic pocket of p66 polymerase domain of HIV-1 RT.[1] Molecular docking of **1**-**4** against HIV-1 RT (PDBID: 3DRP) was carried out using diverse AutoDock 4.0 tools according to the specified instructions.[3]



Compounds **1**-**4** were synthesized in 70-90% yields. The **1**-**4**-HIV-1 RT docked complexes were analyzed on the basis of lowest binding energy values and hydrogen/hydrophobic interaction analyses. Results showed that **2** -HIV-1 RT complex has more stable behavior with the best energy value (-12.80 Kcal/mol) as compared to others docking complexes in the Table.

Table. Binding affinity (ΔG, Kcal/mol) of spirocyclopropyl oxindoles **1**-**4** in complex with HIV-1 RT.

Ligand	**1**	**2**	**3**	**4**
ΔG	-12.45	-12.80	-12.44	-12.40

The molecular docking analysis predict that **1**-**4** form a hydrogen bond at specific residue Lys103 with target protein. The amide carbonyl group of **1**-**4** interacts with Nα of Lys103 with hydrogen bonds length from 2.93 to 3.02 Å. **Conclusion:** The use of the molecular docking approach identified a potential novel spiroocyclopropil oxindoles with affinity against the HIV-1 RT enzyme.

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EFFLUX PUMP INHIBITORS REVERT DRUG RESISTANCE IN MDR-MYCOBACTERIUM TUBERCULOSIS STRAINS

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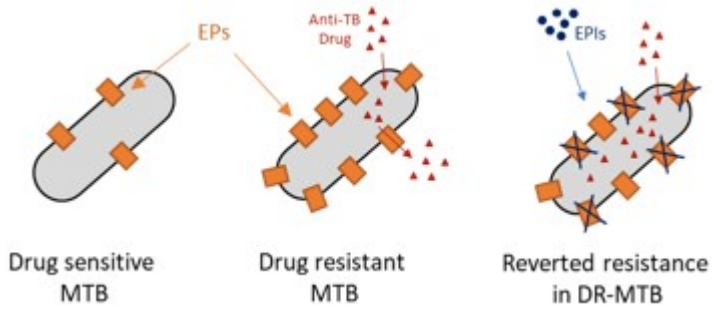
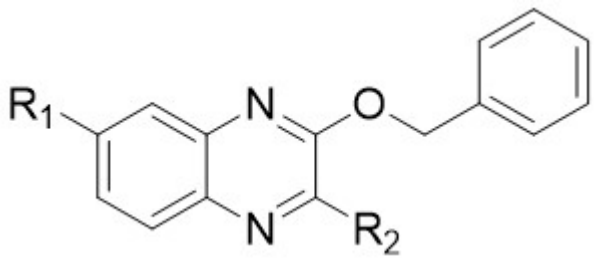
Mycobacterium Tuberculosis (MTB) is nowadays the first cause of death from a single agent, according to the World Health Organization (WHO). It can affect the lungs causing pulmonary tuberculosis (TB), or other organs, causing extrapulmonary TB. In recent years, about 10 million people fell ill with TB every year and in 2018 about 1.2 million TB deaths were registered worldwide. [1] Drug-resistant MTBs continue threating global health with an increasing number of MTB strains which become drug resistant, multi drug-resistant or extensively drug-resistant (DR, MDR and XDR respectively). [1] MTB strains develop spontaneous gene mutations that provide the bacteria resistance to the anti-TB drugs. Reduction of drug affinity for the target is caused by mutations in the gene coding for the drug target. [2] Continued exposure of MTB cells to anti-TB drugs also results in upregulation of the efflux pump system genes, leading to an increased number of efflux pumps (EPs) on the cell membrane. [2] Efflux pump inhibition represents a valid strategy in anti-TB research: partial suppression of the drug extrusion results in intracellular therapeutic concentration restored or even increased.

A new series of 3-phenoxymethyl-quinoxaline derivatives (PMQs) has been designed, synthesised and investigated as extrusion pump inhibitors (EPIs) against MDR-MTB strains. Nine clinical strains of MTB, two non-tubercular mycobacteria (NTM) clinical strains, and *M. Tuberculosis* H37Rv as reference, were used in REMA assays, testing first and second line antituberculosis drugs both in presence and absence of our PMQs, evaluating how EPIs can impact the drug MICs (minimal inhibitory concentrations) values, and therefore the activity.

The different resistance levels tracked in the clinical strains have been reduced by EPIs and in several cases the susceptibility was completely restored. The results obtained in this study indicated that the intrinsic cell-efflux activity significantly contributes to the overall resistance in resistant clinical isolates of MTB and NTM, and that the inhibition of efflux pumps by the PMQs can enhance the clinical effect of antibiotics.





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DE NOVO DESIGN OF MACROCYCLIC PEPTIDES TARGETING THE PD-1/PD-L1 INTERACTION

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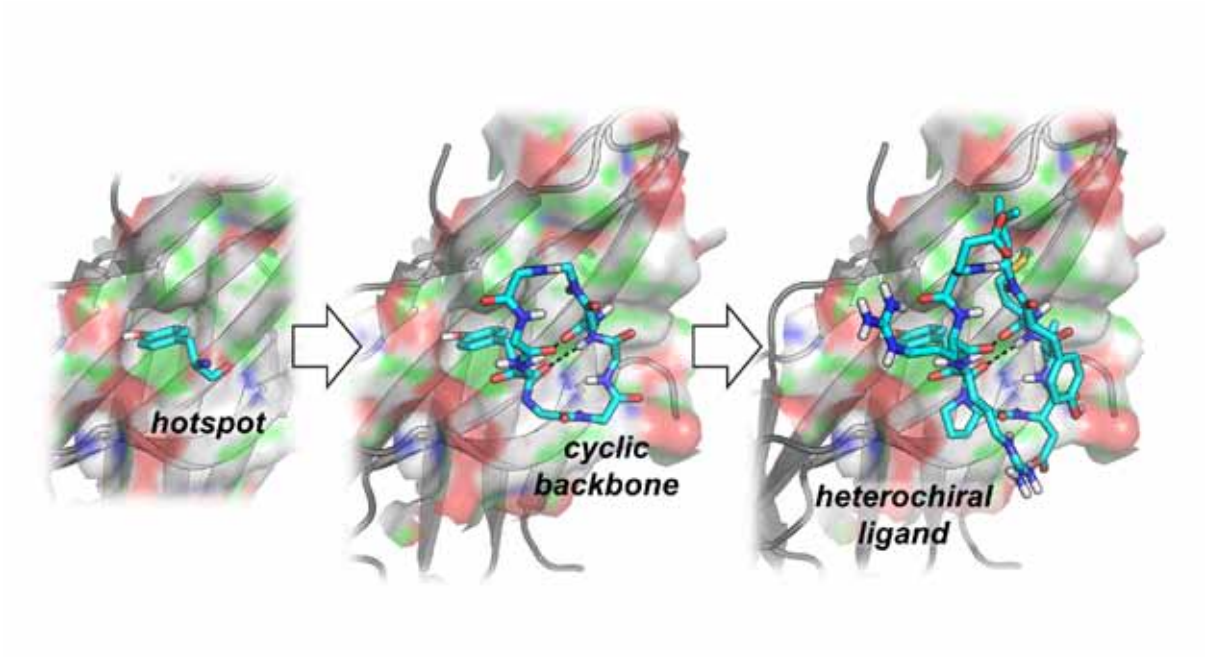
Peptides, together with antibodies, are potent biochemical tools capable of modulating difficult protein-protein interactions. However, current structure-based methods are largely limited to natural peptides and are not suitable for designing target-specific binders with improved pharmaceutical properties, such as macrocyclic peptides. In this work, we report a general framework that leverages the computational power of Rosetta for large-scale backbone sampling and energy scoring, followed by side-chain composition, to design heterochiral cyclic peptides that bind to a protein surface of interest. To showcase the applicability of our approach, we identified two hits (**PD-i3** and **PD-i6**) that target PD-1, a key immune checkpoint, and work as protein ligand decoys. A comprehensive biophysical evaluation confirmed their binding mechanism to PD-1 and their inhibitory effect on the PD-1/PD-L1 interaction. Finally, elucidation of their solution structures by NMR served as validation of our *de novo* design approach. We anticipate that our results will provide a general framework for designing target-specific drug-like peptides.



AN AEROSOLIZED ANTIMICROBIAL COMBINATION FOR THE TREATMENT OF PULMONARY Pseudomonas aeruginosa INFECTIONS

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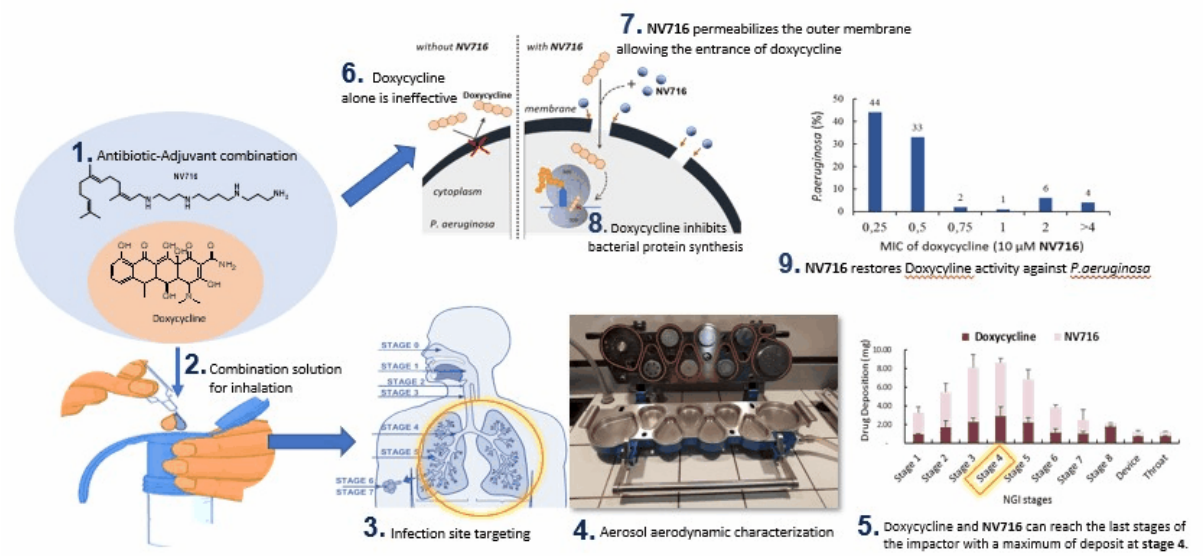
During the last decades, multiple approaches have been developed to combat bacterial resistance. One of them involves combination therapies of existing antibiotics with potentiating adjuvants, reempowering the activity of the antibiotic against resistant strains by improving the permeability or suppressing antibiotic efflux. Once co-administered with an antibiotic these peculiar chemosensitizers “suppress” the resistance and “improve” the antibiotic activity. Currently, many drug delivery systems are used and aim for an optimal drug administration by minimizing drug degradation, decreasing its side effects and increasing its bioavailability. In this context, pulmonary route has become an attractive pathway for infections treatment.

The aim of our study is to develop and evaluate a new potent pharmaceutical form intended for pulmonary administration to defeat cystic fibrosis, based on a combination of an antibiotic (Doxycycline) with an adjuvant: a polyaminoisoprenyl derivative NV716 allowing restoration of doxycycline efficacy against *P.aeruginosa* strains naturally resistant to doxycycline. The proof of concept of such a combination has been previously verified in *vitro* on various *P. aeruginosa* strains. (1)



Here we report the characterization of three different aerosols: doxycycline alone, NV716 alone and doxycycline/NV716 combination, using the Next Generation Impactor (NGI) (EP, USP). Aerosols evaluation was carried out according to different concentration, duration of nebulization and nebulizers: Pari LC Plus, Pari Sprint (breath-enhanced jet nebulizers) and the Pari Eflow (vibrating mesh nebulizer). The droplet size distributions and aerosol efficiency were expressed in terms of Mass Median Aerodynamic Diameter (MMAD) and Fine Particle Fraction (FPF). Results showed MMADs (3,4-4,4 µm) in accordance with the standards recommended for therapeutic aerosols (< 5 µm) (EP, USP) suitable for deep lung deposition, with a high FPF (> 50 %) required to maintain drug level above its Minimal Inhibitory Concentrations (MIC) at the infection site. Furthermore, it has been demonstrated that the delivery of the combination is in high amounts of both compounds required to lead to an efficient antibacterial activity. An air-jet nebulizer was found superior to a vibrating-mesh technology, for the delivery of our formulation due to its high performance and rapid nebulization.(2)

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IDENTIFICATION OF AMENTOFLAVONE AS EPIGENETIC INHIBITOR IN INC5 STRAIN OF TRYPANOSOMA CRUZI

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The parasite *Trypanosoma cruzi* etiologic agent of Chagas disease (or American trypanosomiasis). A neglected tropical disease (NTD), where it not only represents a health problem, also is a global economic problem. It is estimated that there are between 6 and 7 million people infected in the world, which represents the loss of 662,000 disability-adjusted life years (DALY), which represent $29,385,250 on economic loss per year. Classical drug treatment uses benznidazole and nifurtimox, molecules with dangerous side effects. TcSir2rp3 is a novel epigenetic target of *T. cruzi* involved in processes of regulation, differentiation, and response to the environment.

In this work, TcSir2rp3 protein was studied with a consensus-based drug discovery approach (ligand-based and structure-based). A database with 10,432 compounds like the active compounds (previously reported) and the natural substrate of the enzyme was screened, resulting in the proposal of possible trypanocide candidates.

Finally, the most promising candidate (amentoflavone) was selected and evaluated. This was done using an *in vitro* model with the INC5 strain of *Trypanosoma cruzi*, demonstrating its trypanocide activity (20 µM). (1)

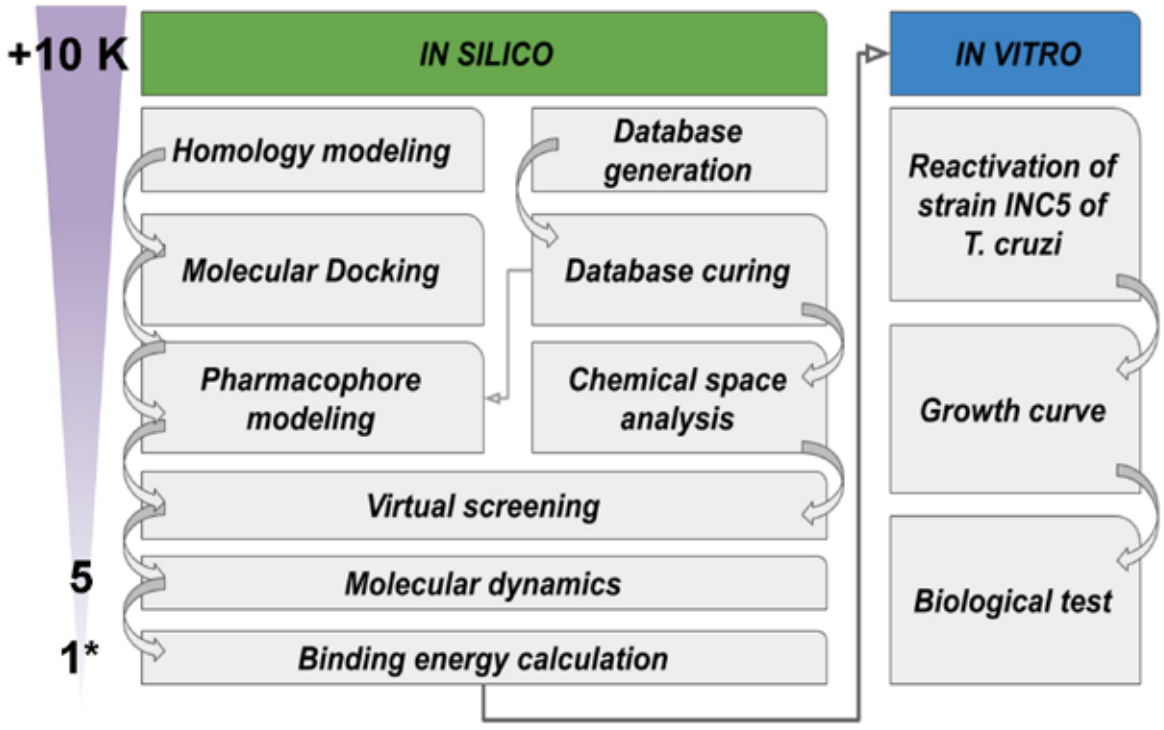

Figure 1. Workflow for the study of the TcSir2rp3 of *T. cruzi*.
* *In vitro* evaluated compounds.

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DISCOVERY OF NOVEL ANTIMICROBIAL LEADS BY DOCKING-BASED VIRTUAL SCREENING

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Antibiotics were considered "Super Drugs" when first discovered in 20th century. Since then, antibiotics have been used as first-line treatments for many bacterial infections. However, over the years, bacteria have developed resistance to these antibiotics, thereby making treatment of antibiotic-resistant bacterial infections difficult. DNA gyrase is a type IIA topoisomerase which catalyses changes in DNA during replication by introducing negative supercoils and, hence, is a well validated drug target for antibacterial drug discovery. Currently, fluoroquinolones and aminocoumarins classes of antibiotics act by inhibiting bacterial DNA gyrase. However, fluoroquinolones class of antibiotics is used for gram-negative bacterial infections and have limited activity against gram-positive bacteria. Similarly, novobiocin, an aminocoumarin class of antibiotics, was initially clinically approved but was later withdrawn from the market due to safety issues and poor pharmacological properties.

Computer-aided drug design has emerged as an important tool for drug discovery in last decade. Therefore, in the proposed work, we utilised docking-based virtual screening approach to identify novel DNA gyrase inhibitors. In this study, a database containing 7 million molecules was subjected to an *in-silico* docking-based virtual screening workflow against DNA gyrase and 38 molecules were selected for the evaluation of antimicrobial activity. In antimicrobial screening, four non-fluoroquinolone class of compounds were identified which showed minimum inhibitory concentration value of 0.5-8 µg/mL against twelve strains of bacteria.

In future, all four lead compounds will be tested for their DNA gyrase inhibitory potential and used as a basis to develop potential DNA gyrase targeted antibiotics.

CONSENSUS VIRTUAL SCREENING OF A LARGE CHEMICAL LIBRARY TO IDENTIFY INHIBITORS OF DNA METHYLTRANSFERASE

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The DNA methyltransferases (DNMT) comprise a family of different isoforms enzymes that have a central role in epigenetic gene regulation. Recently studies reveal that DNMTs are promising targets for the treatment of different types of cancer and other diseases [1-2]. In the present work, we determine possible computational drug candidates for DNMT3A from an extensive Chemspace database, which contains 9436 compounds. We carry out a virtual screening analysis based on two principal methods: molecular docking method [3-4] wielding two different software packages (MOE and LeDock) employing the crystallographic information available in RSCB Protein Data Bank; and Statistical-based database fingerprint method (SB-DFPs) [5]. After filter the results achieved, we obtained 80 compounds called compounds selected from consensus analysis (CS). The CS was subsequently brought under in silico analysis to evaluate some Drug like descriptors using the DataWarrior package. In conclusion, we obtained 32 potential computational candidates as inhibitors of DNMT3A. Figure 1 summarizes the overall strategy. This work suggests evaluating the inhibitory activity of these compounds on DNMT3A with in vitro and in vivo studies.

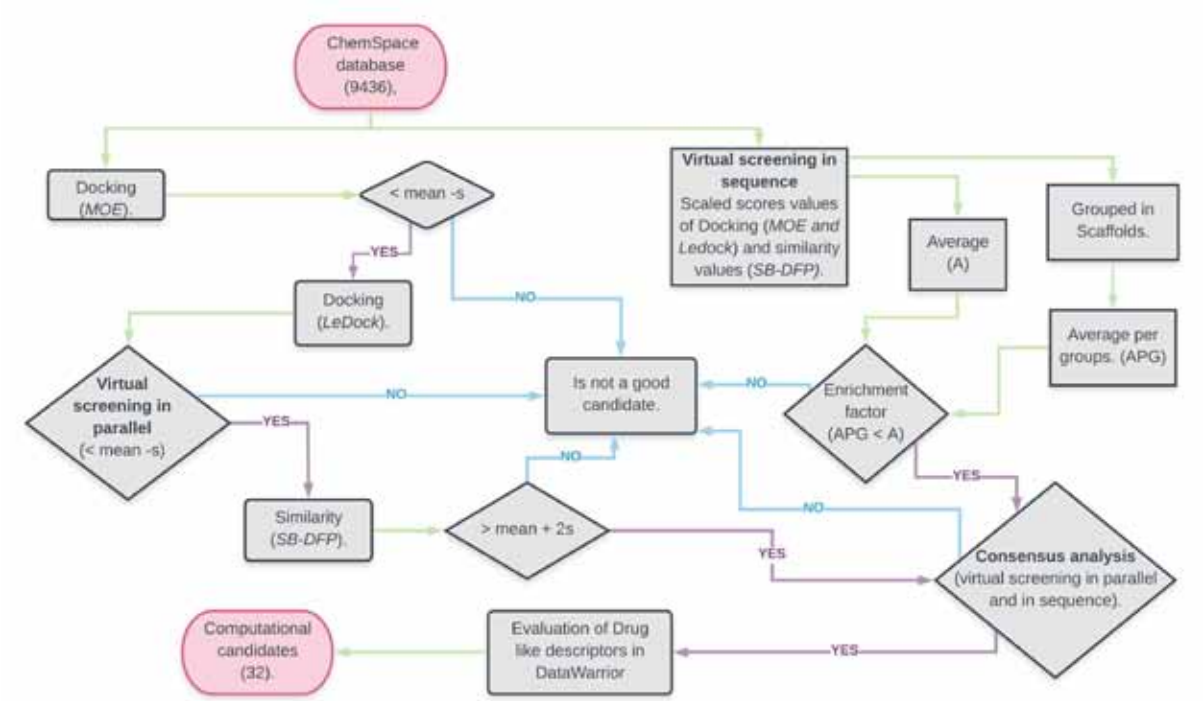

Figure 1. Workflow of the virtual screening pursued in this work.

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SYNTHESIS OF NEW ADAMANTANE-MONOTERPENE CONJUGATES AND INVESTIGATION OF THEIR ANTIPOX ACTIVITY

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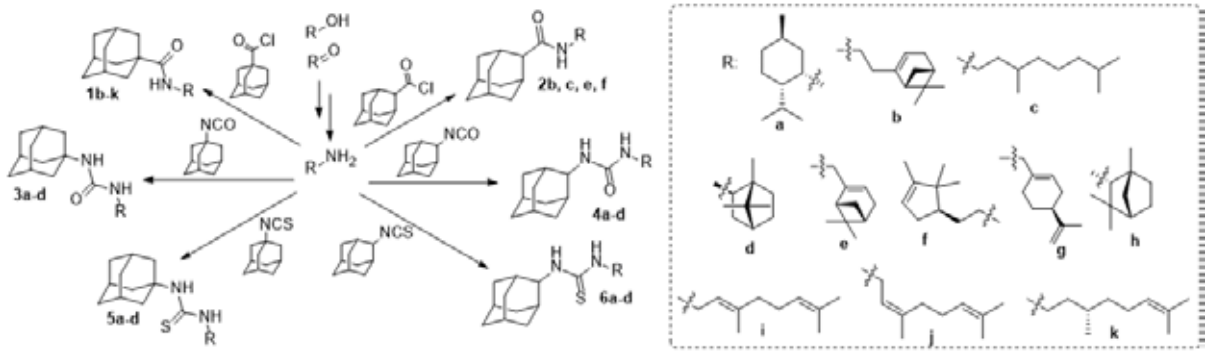
Although in 1980 WHO declared eradication of natural smallpox, Independent Advisory Group on Public Health Implications of Synthetic Biology Technology Related to Smallpox report to the WHO Director-General noted the need to continue developing new low molecular weight agents against variola virus because of a number of reasons, such as discounted vaccination, possibility of smallpox spreading from permafrost soils, bioterrorism, potential danger of other orthopoxviruses circulating in animal population [1].

Some adamantane derivatives are known to possess antiviral activity, mainly against the influenza virus, but it was found that few adamantane derivatives showed activity against vaccinia virus [2]. From the other hand, monoterpene derivatives demonstrated various biological activities, such as antibacterial, antiviral and others, moreover, some camphor derivatives were found to be active against vaccinia virus as well [3]. In attempt to find new agents against orthopoxviruses we synthesized a number of conjugates combining adamantane and monoterpene derivatives via different linkers.

A number of amides of 1- and 2-adamantane carboxylic acids was synthesized starting from corresponding monoterpene alcohols and ketones via amine formation. Interaction of monoterpene amines with corresponding carboxylic acid chlorides lead to amides **1b-k, 2b, c, e f**. For investigation of influence of amide fragment on antipox activity, some ureas and thioureas were synthesized. For this purpose, isocyanates and isothiocyanates containing 1- and 2-substituted adamantane fragments were obtained from corresponding amines or carboxylic acid chlorides.



Amides **1b-k, 2b, c, e f** were tested against orthopoxviruses, they were shown to be active against vaccinia virus with SI from 5 to 1123. A most active amides (**1b**, **1e**, **2b**, **2e**) were investigated against cowpox virus and ectromelia virus, with SI being from 30 to 406 and from 39 to 707 respectively [4].

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SYNTHESIS OF NEW SPIROCYCLIC AZETIDINES AND EVALUATION OF THEIR ACTIVITY AS ANESTHETIC AGENTS, ANALOGUES OF BUPIVACAINE

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The need for new and non-toxic anesthetics is continuously high. To overcome such often side effects as myocardial ischemia and other cardiovascular disorders researchers do need to find new molecules, modulators of ion channels. In the progress of drug discovery, several valuable concepts, such as scaffold hopping, “Escape from Flatland” and conformational restriction, were introduced and already gained great recognition in the scientific community. These conceptual developments rely, in particular, on the generation of new 3D-shaped Fsp3-rich building blocks, thus making these molecules attractive targets for the synthesis and exploration of new non-toxic ion channel modulators.

STRUCTURE-BASED IDENTIFICATION OF P-GLYCOPROTEIN INHIBITORS FROM NATURAL SOURCES

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ATP-binding cassette (ABC) transporters are a large family of proteins involved in membrane transport of a wide variety of substrates. Among them, ABCB1, also known as MDR-1 or P-glycoprotein (P-gp), is the most characterized. By exporting xenobiotics out of the cell, P-gp activity can affect the ADME properties of several drugs. Moreover, P-gp has been found to mediate multidrug resistance in cancer cells. Thus, the inhibition of P-gp activity may lead to increased absorption and/or intracellular accumulation of co-administered drugs, enhancing their effectiveness.[1]

While some attempts to perform structure-based discovery of P-gp inhibitors have been carried out, many of the currently known P-gp inhibitors were characterized exploiting ligand-based approaches, due to the lack of well-refined human crystal structures.[2]

However, recently human cryoEM structures, with and without co-crystallized ligands, have been resolved, giving more structural insights into the different conformations of the protein.[3]

Using the human cryoEM 3D structure of the P-gp in the inhibitor-bound intermediate form (PDBID: 6qee), we virtually screened approximately 200'000 commercially available natural compounds from the ZINC database.[4] Raccoon2 and AutoDock Vina were used for performing the molecular docking simulations.[5]

To build a model able to discriminate between substrate and inhibitors and to filter the virtual screening results, we also docked a dataset of 3250 compounds with known activity, including P-gp inhibitors and substrates, as well as inactive molecules. The Autodock Vina scoring function was shown to be able to enrich known binders over not binders, with more than 80% of the top quarter of docking results containing known binders. Then, 6 molecular descriptors available in DataWarrior were used to perform structural similarity clustering of known inhibitors and substrates independently.[6] Then, the most representative molecule for each cluster was used to generate 3D common pharmacophores, using their best docked pose in the P-gp binding site.[7] The pharmacophores were then used to re-score virtual screening results, and molecules matching all the inhibitors pharmacophores and none of the substrates pharmacophore were chosen for visual inspection.

With this consensus approach, we were able to identify 10 potential candidates which will be tested for their ability to inhibit P-gp.

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IDENTIFICATION OF PEPTIDIC Rpt5(10) PROTEASOME ACTIVATOR - BINDING SITES BY ALANINE SCAN

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The accumulating evidence in the last decade indicates the 20S proteasome, without its regulatory particle(s), as the major degradation machinery of various disordered or damaged proteins [1]. The malfunctioning of this system can lead to aggregation events, that are hallmarks of many proteinopathies, including neurodegenerative diseases and diabetes. Therefore, searching for activators of this enzyme is an important pharmaceutical target. Short peptides with sequences derived from natural protein activators represent one of the organic compounds class which can be used as such stimulating agents.

In order to such peptide activators become promising pharmaceuticals, knowledge about the importance of amino acids forming the sequence of a given peptide, as well as the characteristics of interactions with a given enzyme and understanding the mechanism of activation, is needed. One of the ways to understand these complex interactions is to obtain a library of compounds - activator analogs - based on the alanine scan. Alanine scanning is used to identify peptide-protein interaction sites by systematically substituting functional groups along the peptide chain with a methyl group.

In search for crucial motif responsible for the 20S activation we synthesized and tested library of peptide Rpt5(10) analogs by alanine scan technique. Rpt5(10) peptide is an activator of proteasome 20S which sequence is derived from natural 19S regulatory particle [2]. The obtained results will be presented and discussed.

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OPTIMIZED SYNTHESIS OF 7-AZA-INDAZOLES BY DIELS–ALDER CASCADE AND ASSOCIATED PROCESS SAFETY

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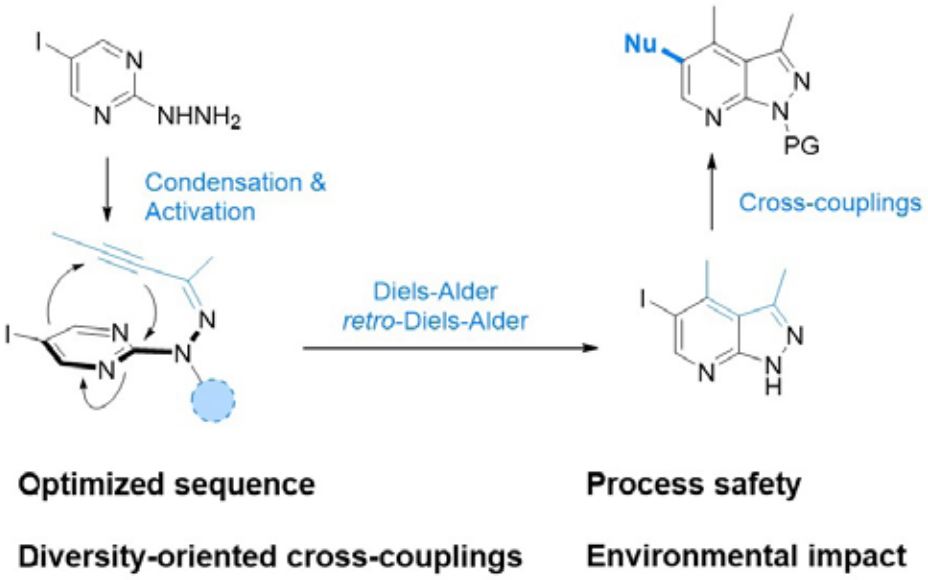
Although pyrimidines are not among the most reactive partners in intramolecular inverse-electron demand [4+2] reactions with alkynes,[1] they could be activated under mild and practical conditions, leading to fused nitrogen-containing heterocycles.[2] We report an optimized synthesis of a 5-iodo-7-*aza*-indazole by a one-pot Diels-Alder cascade that starts from a pyrimidine substituted in the 2-position by an (alkynyl)hydrazone. The safety of the process and the environmental impact were thoroughly evaluated. Eventually, a selection of cross-coupling reactions were studied and allowed the introduction of carbon- and nitrogen-based nucleophiles at the C5-position in good to excellent yields.[3]



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CHIRAL AND NON CHIRAL N-BOC PROTECTED 7-AZAINDOLINES IN BATCH AND FLOW

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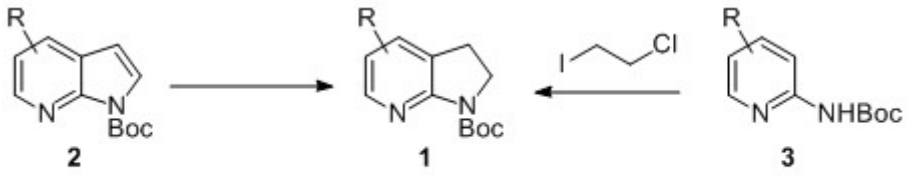
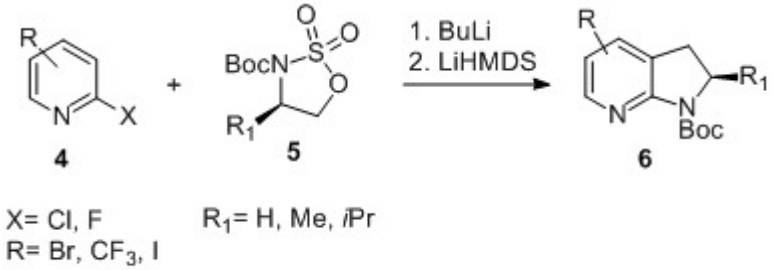
7-Azindolines are a common structural motif in modern drugs.[1] Despite this fact only a couple of general methods have been developed to synthesise these compounds. Especially *N*-Boc protected 7-azaindolines with a variety of halogenated substituents have only been reported a few times mainly through reduction of Azaindoles **2**[2] or the alkylation of *N*-Boc protected 2-aminopyridines **3** (Scheme 1).[3]



Scheme 1.

In our ongoing efforts to make new chiral building blocks for the pharmaceutical and agricultural industries as well as academia we have developed a general method from commercially available pyridines **4** and (*R*) or (*S*) *N*-Boc 1,2,3-oxathiazolidine-2,2-dioxides **5** to make chiral and non chiral *N*-Boc 7-azaindolines **6** in a 2 step procedure (Scheme 2, only one enantiomer shown for clarity).[4]



Scheme 2.

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DIVERSITY-ORIENTED LIBRARY SYNTHESIS: LATE-STAGE TRANSFORMATIONS OF THE 4-OXO-2-BUTENAMIDE SCAFFOLD

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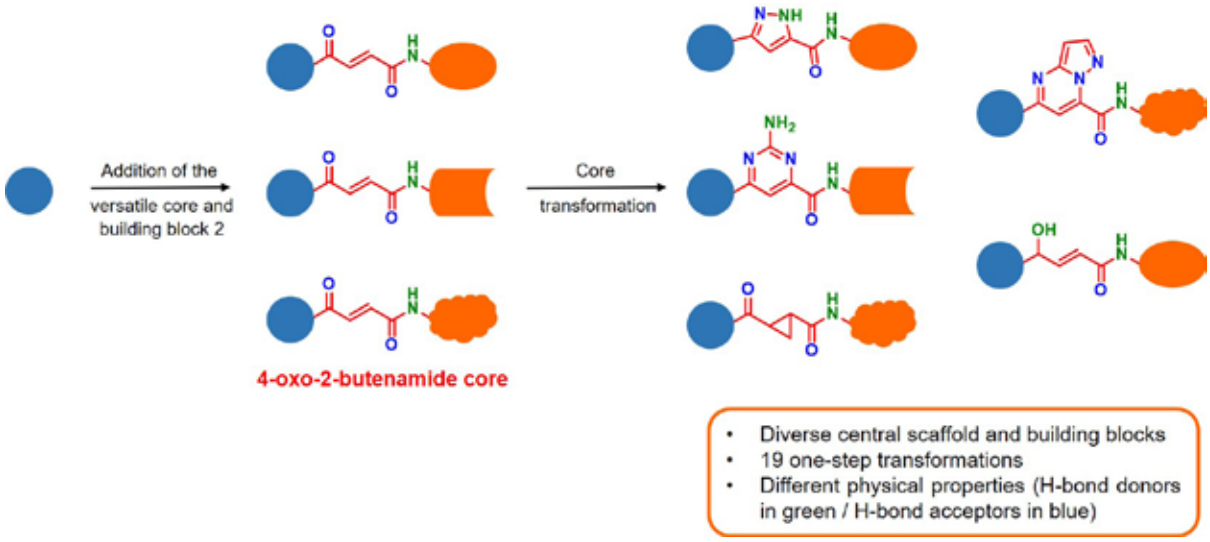
Drug discovery programs often rely on screening of compound libraries to find start points. It is crucial that these libraries consist of structurally diverse drug-like compounds (various molecular weight, lipophilicity, H-bond donors/acceptors, geometries, etc.) to maximise screening success rates. The development of a late-stage diversity-oriented library synthetic protocol that allows a series of compounds to be transformed into a range of drug-like scaffolds would provide an efficient route to the production of libraries of greater structural diversity. To illustrate this concept, we selected the 4-oxo-2-butenamide scaffold as a suitable precursor scaffold that can undergo a series of different transformations towards diverse drug-like cores. We have developed a range of transformations that can be applied to the central 4-oxo-2-butenamide moiety. So far, nineteen different reactions including 3,4-cyclisations, 1,4-cyclisations, 1,4-additions and reductions have been developed. These reactions yield the desired product in one step with good yields, providing a small library of drug-like compounds in an efficient manner. The scope of each reaction is being evaluated using pooled chemistry. These late-stage transformations will be applied to library design or structure-activity relationships for drug discovery. Finally, the produced library will be tested against selected biological targets for hit identification.



A NEW GROUP OF PIPERAZINE DERIVATIVES AS POTENTIAL HISTAMINE H3 RECEPTOR LIGANDS WITH ANTIOXIDANT PROPERTIES

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Paying attention to the activity of highly active and selective histamine H_3 receptor (H_3R) ligands that were previously presented by our group, we modified a series of ligands with “east- & west-ends” modifications. Thereof, 9 newly obtained compounds showed moderate affinity at the human H_3R (hH_3R), with best compound QD18 of K_i = 518 nM at hH_3R. Docking to an H_3R homology model revealed two possible binding modes, with key interactions retained in both cases. Moreover, despite their moderate affinity for histamine H_3 receptors, selected compounds were assessed for their antioxidant properties. Among others, QD 13 (hH_3R K_i = 592 nM) showed the highest antioxidant properties, exhibiting 50-60% of ascorbic acid activity at two different concentrations. Therefore, QD13 emerged as a promising candidate for further biological tests.

Acknowledgements

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DUAL AROMATASE INHIBITORS AND SERMS AS POTENTIAL AGENTS TARGETING BREAST CANCER

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At present, two main strategies are employed for the treatment of estrogen-dependent breast cancer, which act on two different targets. Aromatase inhibitors (AIs) block the activity of aromatase enzyme,hampering the endogenous synthesis of estrogens, while SERMs bind to estrogen receptors acting as agonist or antagonist depending on the different tissues. The observation that some metabolites of tamoxifen (SERM prototype) are able to inhibit aromatase enzyme and, at the same time, to bind estrogen receptors with high affinity[1] opened the way for the design of new dual-acting compounds, single molecules that could act on two different targets involved in the same pathology. Starting from the structure of a literature lead compound (inspired by tamoxifen metabolites) that proved to be a potent aromatase inhibitor with good affinity for estrogen receptors,[2] a series of compounds carrying different structural modifications have now been synthesized (Fig.1). The compounds were tested on aromatase enzyme and estrogens receptors and they showed different biological profile, being some of them endowed with dual activity.

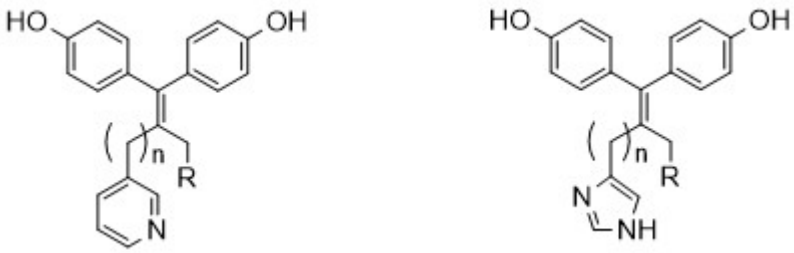

Fig.1: General structures of synthesized compounds.

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SYNTHESIS, OPTICAL PROPERTIES AND BIOLOGICAL ACTIVITY OF VARIOUSLY SUBSTITUTED-2H-PYRAZOLO[4,3-c]PYRIDINES

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Pyrazole is a common structural unit in many pharmaceuticals and a central axis of numerous ongoing studies devoted to the synthesis and biological evaluation of novel pyrazole moiety-bearing molecules. Annelated pyrazoles are of particular interest as they constitute the core of several well-known drugs, including Sildenafil, Zaleplon and Allopurinol. Among the vast variety of up to now developed biologically active annelated pyrazole derivatives, synthetically demanding 2*H*-pyrazolo[4,3-*c*]pyridines are still relatively understudied.

Herein we present synthesis of novel variously substituted 2*H*-pyrazolo[4,3-*c*]pyridine derivatives, which are easily accessible from various pyrazolidin-3-ones. The compounds were evaluated for their optical properties and cytotoxicity *in vitro* against a small panel of normal and cancer cell lines. The compounds exhibited favorable cytotoxicity, with GI50 values in the micromolar range and selected ones were further studied to assess their mode of action.

Acknowledgements

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POSITIVE ALLOSTERIC MODULATORS OF CANNABINOID RECEPTORS MODULATE THE NEUROPROTECTIVE ACTIVITY OF A DUAL CB1R/CB2R ORTHOSTERIC AGONIST

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A great amount of preclinical studies highlighted that compounds targeting the ECS could be useful for developing novel therapies against neurodegenerative disorders. Endocannabinoid signalling may be beneficial because of its impact on the attenuation of neurotoxicity through neuronal CB1R, limiting glutamate release. Furthermore, the activation of CB2R decreases microglial-derived neuroinflammation, modifying the rate between pro- and anti-inflammatory cytokines released by these cells. Despite their theoretical usefulness, the chronic use of CB1R and CB2R orthosteric agonists has several disadvantages, limiting their usefulness as clinically relevant drugs. In particular, CB1R orthosteric agonists induce psychotropic effects, strong mood alteration, acute psychosis, and cognitive and motor impairments. On the contrary, CB2R ligands, which do not produce psychotropic effects, appear to be promising drugs for treating several neuro-inflammatory diseases. However, their predominance on immune cells might cause immunosuppression. Positive allosteric modulators (PAMs) might represent a promising approach to achieve the potential therapeutic benefits of orthosteric CBR agonists increasing their activity and limiting their adverse effects. The goal of the present study was to determine whether the positive allosteric ligands **Gat 229** [(a)] and **EC21a** [(b)] of CB1R and CB2R respectively can modulate the activity of the potent dual CB1R/CB2R orthosteric agonist **FM6b** (Figure 1) on neuroinflammation and excitotoxic damage due to an excessive glutamate release.

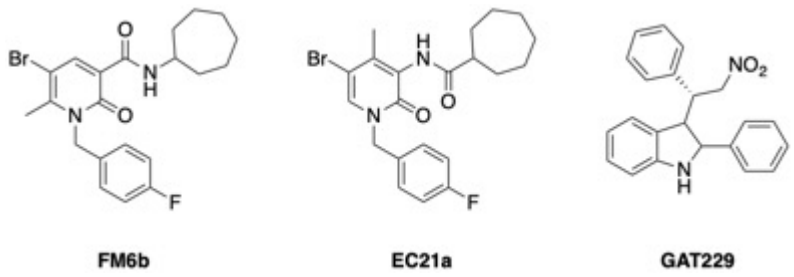

Figure 1. Chemical structure of **FM6b**, **EC21a** and **GAT229**.

The obtained results indicate that the administration of **FM6b** with either **EC21a** or **GAT229** could represent a promising therapeutic approach for the treatment of neurodegenerative disorders.

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SYNTHESIS OF ANTI-INFLAMMATORY AND PRO-APOPTOTIC CYCLOPENTENONE PROSTAGLANDIN MIMICS

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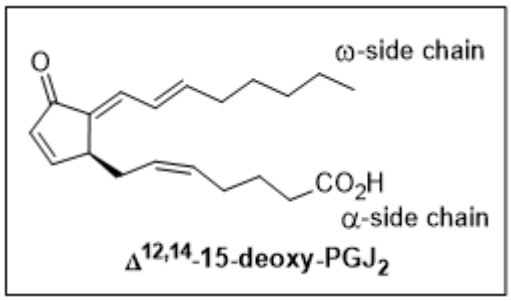
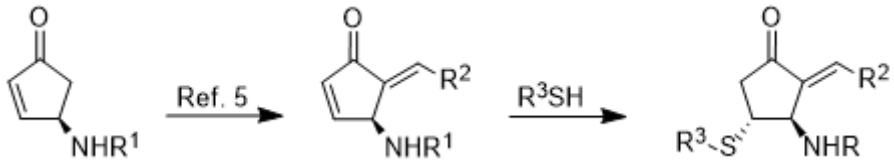
Prostaglandins (PGs) are naturally occurring structures containing 20 carbons, and are derived from arachidonic acid.[1] They are local hormones which play key roles in controlling a multitude of physiological properties.[2] It has been indicated that cyclopentenone PGs inhibit nuclear factor κ-B-dependent transcription (NF-κB) which is pivotal in promoting inflammation and controlling cell proliferation and survival.[3] Upregulation of NF-κB is also associated with many tumour types and often leads to poor prognosis for treatment of certain cancers. Several syntheses of PGs shown to target this transcription factor exist,[4] however they are challenging approaches which generally do not allow for the facile synthesis of analogues.



With this in mind, the synthesis of 4-aza cross conjugated cyclopentenones, inspired by the PG $\Delta^{12,14}$ -15-deoxy-PGJ_2, is described. The use of a Baylis-Hillman type reaction[5] to install the exocyclic alkene is discussed. Using this technique, a range of aldehydes may be used to mimic the ω-side chain of natural prostaglandins.[6] The presence of the 4-amino substituent allowed for derivatisation of the compounds, installing the α-side chain. Research in to masking the electrophilic atom with a sulphur atom will also be discussed.



In addition, the library of 4-amino functionalised cyclopentenones along with their cysteine adducts were tested for inhibition of NF-κB.

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PHOTOCHEMICAL SYNTHESIS OF BENZIMIDAZOLES FROM DIAMINES AND ALDEHYDES

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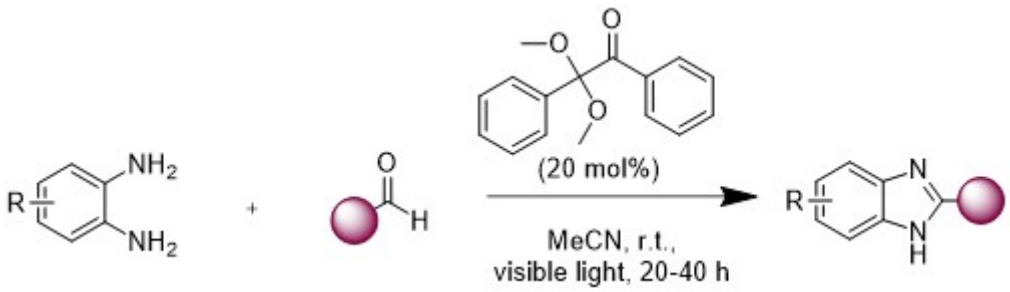
Photoorganocatalysis, the use of small organic molecules and light for the promotion of organic transformations, has provided new reaction platforms and methodologies which are environmentally friendly, since they utilize inexpensive and non-toxic reagents. Benzimidazoles belong to a category of heterocyclic compounds that exhibit various interesting medicinal properties. Our group has developed several methods that harness the power of light through the use of photocatalysts, in order to synthesize molecules with various bioactivities. Herein, we introduce an alternative, direct and metal-free protocol for the synthesis of benzimidazoles from diamines and aldehydes, utilizing 2,2-dimethoxy-2-phenylacetophenone as the photoinitiator and irradiation from household lamps. Cyclization of a variety of aldehydes has been achieved and the corresponding products were afforded in good to high yields.



SYNTHESIS OF NEW BIOLOGICALLY ACTIVE CARBAMATES

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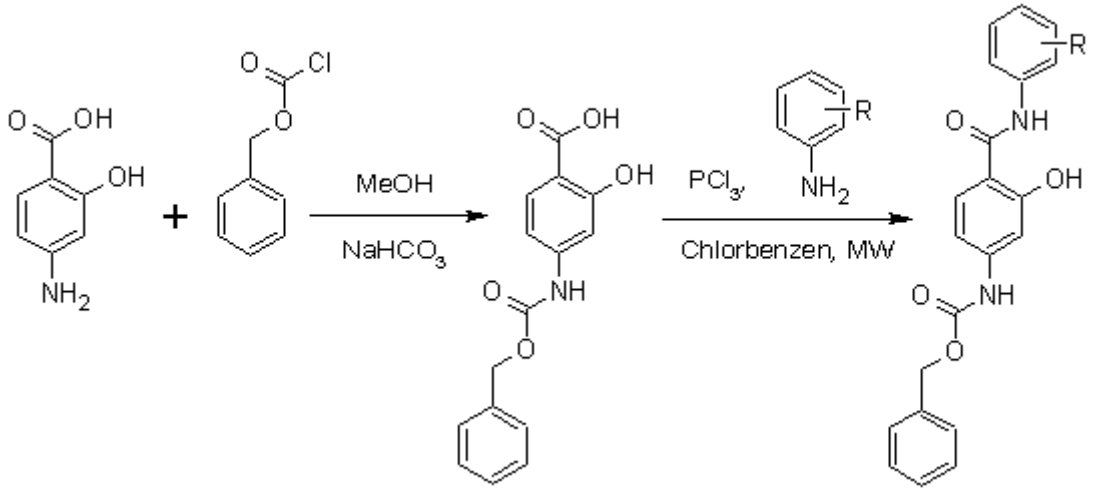
Cholinesterase inhibitors catalyze the hydrolysis of acetylcholine, may increase acetylcholine levels in the brain, thereby improving the cognitive symptoms of Alzheimer's disease. Symptomatic drugs used in the treatment of Alzheimer's disease include carbamates with the ability to inhibit cholinesterases, which were studied in this research. The synthesis consisted of two steps, in the first step the primary amino group of para-aminosalicylic acid was protected by benzyl chloroformate. In the second step, anilides were formed from the intermediate compound and the corresponding anilines in a microwave reactor. All products were identified by using available spectral methods (IR, ^{1}H-NMR, ^{13}C-NMR, MS) and submitted for the evaluation of biological activity.



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FUNCTIONALIZED UPCONVERTING NANOPARTICLES BY LIGHT INDUCED TETRAZOLE-ALKENE 1,3-DIPOLAR CYCLOADDITIONS FOR ANTITUMOR PHOTODYNAMIC THERAPY

Simón de la Fuente Silvia (1,7), Ortiz Rojano Laura (1,7), Jingke Yao (2,7), Ortgies Dirk H. (2,6,7), Martín-Rodríguez Emma (2,3,7), Sanz-Rodríguez Francisco (4,7), Ribagorda Lobera María (1,5,7)

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Keywords: Photoclick Chemistry, 1,3-Dipolar cycloaddition, Nanoparticles, Photodynamic Therapy (PDT)

The light induced tetrazole-alkene 1,3-dipolar cycloaddition[1] has emerged as an efficient synthetic tool for the light-ligation and labelling of biomolecules[2]. Our interest in extending the synthetic applications of this photoclick reaction[3], led us to study the functionalization of rare earth doped upconversion nanoparticles (UCNP) [4] with potential organic photosensitizers (PS) using this 1,3-dipolar reactions. The key step is the clean photolysis of tetrazole into a nitrile imine dipole that upon reaction with the corresponding alkene would give the UCNP-pyrazoline cycloadducts[5].

UCNPs have multiple advantages in their use for both diagnosis and medical therapy. Some of these advantages are the selective conversion of energy from infrared wavelengths (NIR) to shorter NIRs and even at visible wavelengths, which allows working in biological windows (NIR-I and NIR-II), thus avoiding tissue autofluorescence biological and acting as markers and contrast agents for bioimaging[6].

The bioorthogonality of the tetrazole photoclick chemistry has been re-evaluated, exploring the functionalization of nanoparticles with organic-type photosensitizers, capable of generating singlet oxygen ($^1O_2$) under 480 nm excitation and thus study energy transfer processes for their potential use in photodynamic therapies (PDT) using a laser of 808 nm as the irradiation source **Figure 1.**

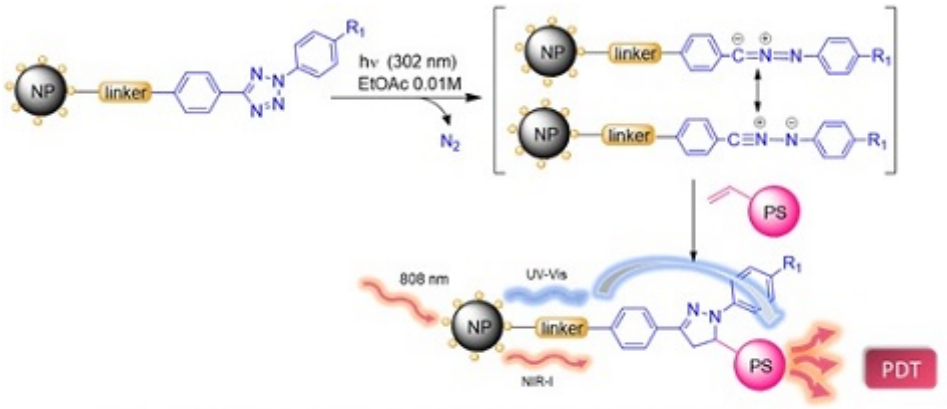

Figure 1. Photoclick reaction with functionalized NP and organic photosensitizers (PS)

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SYNTHESIS AND DESIGN OF 1,4-QUINONE BASED NATURAL PRODUCTS AND DERIVATIVES AS MITOCHONDRIAL COMPLEX I SUBSTRATES AND SUBSTRATE-BASED INHIBITORS

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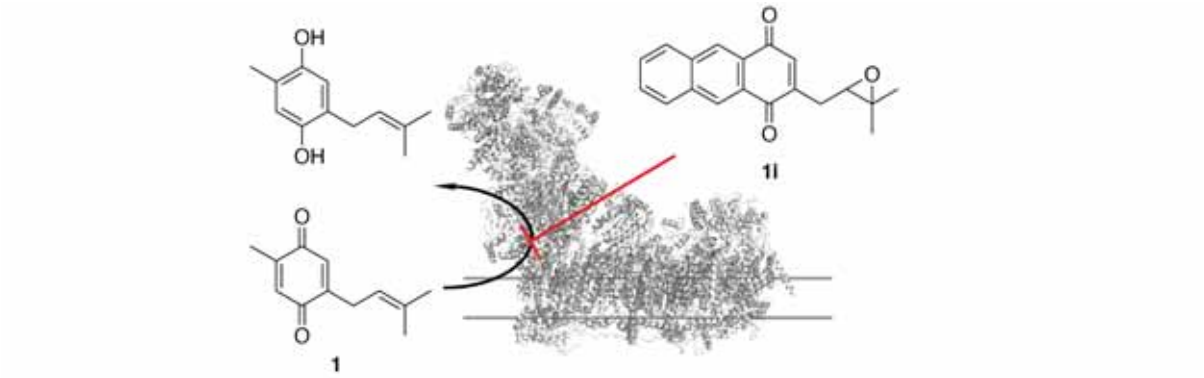

This work has been recently published in *ChemMedChem*.[1]

Mitochondrial complex I (MCI), also known as NADH:ubiquinone oxidoreductase, couples the electron transfer from NADH to ubiquinone and contributes to the formation of the proton motive force in ATP synthesis. MCI is involved in oxidative stress and apoptosis and its dysfunction can lead to neurodegenerative diseases and cancer.[2] Inhibitors targeting the substrate binding site of MCI have the potential to be developed into new antitumor agents.[3-7] However, the access pathway and the binding site of MCI substrates are unknown and the rational design of chemical probes for MCI is challenging.[8] Here we show that 2-methyl-5-(3-methyl-2-butenyl)-1,4-benzoquinone (**1**), a natural product from *Pyrola media*, is an artificial substrate due to its structural resemblance to ubiquinone. Furthermore, structure-activity relationship study led to the discovery of 3-methylbuteneoxide-1,4-anthraquinone (**1i**), a novel substrate-based inhibitor. We found that **1i** has an IC_{50} of 5 μM and exhibited high selectivity for MCI when tested against other quinone-converting enzymes. Importantly, the identified inhibitor was active against selected cancer cell lines in cell-based proliferation assays. Our result demonstrates how substrate-based inhibitor design allowed the transformation of a substrate into an inhibitor. With the capabilities of natural products to access unique therapeutic modalities, our approach could be utilized for the discovery of novel chemical probes for clinically relevant proteins.

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INITIATING DNA-ENCODED LIBRARY SYNTHESIS WITH SOLID-PHASE BOUND DNA BARCODES

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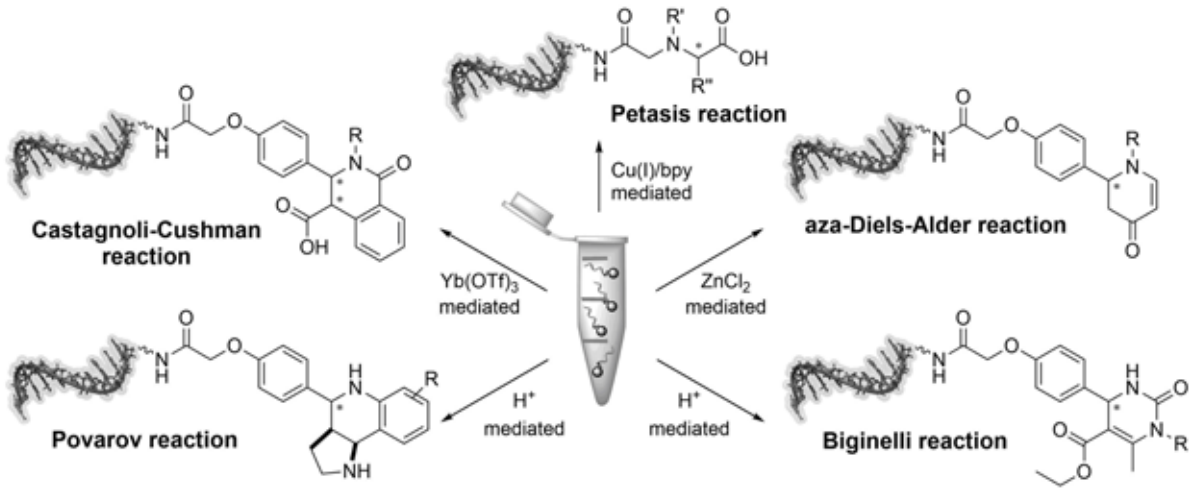
Selection of DNA-encoded small molecule libraries (DELs) is a fast and economic alternative to brute force screening of discrete compound collections. Nevertheless, the preparation of structurally diverse DELs is still a formidable challenge due to the low stability of DNA under many standard reaction conditions in organic synthesis and the requirement to work with aqueous co-solvents in solution-phase DEL synthesis.[1] Alternatively, DEL synthesis is initiated on solid phase-coupled DNA which allows for use of dry organic solvents. A screening campaign assessing the stability of fully protected, solid-phase bound DNA in the presence of 53 metal salts and organic reagents that are commonly used as catalysts in organic synthesis, revealed to our surprise that such DNA tolerated several of the catalysts under mild conditions.[2] Based on these findings, we have selected metal catalysts and reaction conditions that can be used in reactions for initiating DEL synthesis.



To expand the chemical space for DNA-encoded libraries, we established the $Yb(OTf)_3$-mediated Castagnoli -Cushman reaction[3], the $ZnCl_2$-mediated aza-Diels-Alder reaction[2], the CuCl/bpy-mediated Petasis reaction[4] as well as the (*R*)-(-)-BNDHP-mediated Povarov and Biginelli reactions[2]. In all cases, we observed good compatibility of the reaction conditions with a 10mer DNA barcode and we were able to synthesize a collection of DNA-tagged, diverse substituted heterocycles. A novel encoding scheme was developed for tagging these heterocycles.[3]

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DESIGN AND SYNTHESIS OF NEW 3-CYANOPYRIDINES AS SURVIVIN INHIBITORS AND APOPTOSIS INDUCERS

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Overexpression of survivin is usually accompanied by increased cancer aggressiveness in addition to the resistance of cancer cells to chemotherapeutic agents. Accordingly, survivin is considered as an attractive target for developing new hopeful anticancer candidates. Here, a new series of 3-cyanopyridines was synthesized and evaluated for their antiproliferative activity against three human cancer cell lines; prostate carcinoma (PC-3), breast cancer (MDA-MB-231) and hepatocellular carcinoma (HepG2). Compounds **5c** and **5e** displayed promising cytotoxic activity against all the tested cell lines. The safety of these compounds was evaluated by testing on the normal cell line WI-38, showing toxic selectivity towards cancer cells over normal ones. Further studies were done in order to recognize their possible mechanism of action**;** we studied the ability of compounds **5c** and **5e** to induce cell cycle arrest. Both resulted in remarkable induction of cell cycle arrest at the G2/M phase, with an increase in the DNA content in the pre-G1 phase giving an indication of the apoptosis induction. **5c** and **5e** were further subjected to Annexin V-FITC assay in order to estimate their ability to induce apoptosis. Results revealed a marked increase in the early and late apoptotic cells, and an increase in the percentage of necrosis. Moreover, western blotting analysis was performed using different concentrations of **5c** and **5e**. Results revealed significant inhibition of survivin in addition to some other IAP family proteins; Livin, XIAP, and C-IAP1 in a concentration-dependent manner. Docking study of **5c** and **5e** in the active site of surviving revealed that both compounds located in the dimerization site with nearly similar binding poses.

VIRTUAL SCREENING TO UNCOVER POTENTIAL SARS-COV-2 MAIN PROTEASE INHIBITORS IN DARK CHEMICAL MATTER AND FOOD CHEMICALS

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The recent outbreak of the COVID-19 disease caused by the virus SARS-CoV-2 has significantly affected different aspects of human life, has caused more than 700 000 deaths and infected more than 20 million people worldwide. Even though there are several drugs under clinical trials for its treatment, to date, there is no effective drug or vaccine available. The rapidly increasing information regarding the molecular targets of SARS-CoV-2 virus and related coronaviruses allows the application of *in silico* methods for the proposal of drug candidates. A consensus virtual screening of underexplored regions of chemical space is described in this study to contribute to global efforts in the fight of the COVID-19 pandemic. Food chemicals and compounds known as dark chemical matter were analyzed by combining similarity searching with different queries and fingerprints, molecular docking, and ADMETox profiling. Potential inhibitors of SARS-CoV-2 main protease (M^{pro}) were selected, within which folates were found. Moreover, angiotensin II and angiotensin IV showed structural similarity to previously reported potential M^{pro} inhibitors. The full list of virtual screening hits is publicly available. [1]

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EXPLAINING mTOR KINASE INHIBITION BY TWO ATP-SITE INHIBITORS THROUGH QUANTUM BIOCHEMISTRY

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The mammalian target of rapamycin (mTOR), is a ubiquitous serine/threonine kinase that regulates cell growth, proliferation and survival, and is frequently deregulated in cancer.[1] In this work, crystallographic data of mTOR complexed with two ATP site inhibitors: PP242 (also known as torkinib) and PI-103,[2] were optimized through combined quantum mechanics/molecular mechanics (QM/MM) calculations using an MNDO/AM1/PM3 level of calculation for the QM region which include the inhibitors and the YAMBER force field for the MM region.[3] The obtained structures were used to perform quantum biochemistry calculations based on the framework of density functional theory (DFT) and within the molecular fractionation with conjugated caps (MFCC) scheme.[4,5] Our calculations revealed a total interaction energy of -65.70, and -87.30 kcal mol^{-1} between mTOR and PP242 and PI-103, respectively, which are in good agreement with known experimental results. Residues Leu2185, Tyr2225, Ile2237, Trp2239, Val2240, Ile2356 were identified as the main interacting mTOR amino acid residues with interaction energy contributions lower than -4.0 kcal mol^{-1} for both complexes.

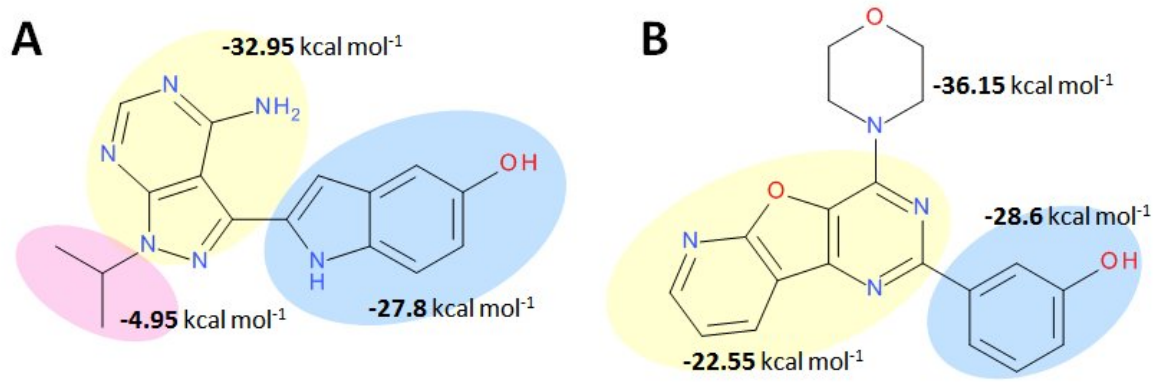

Fig.1. Contribution of moieties of the analyzed ATP site inhibitors to their interaction energies with mTOR enzyme. A. PP242. B. PI-103.

In the case of the inhibitors, their aminopyrazolo[3,4-d]pyrimidin moiety (Fig. 1A) and morpholin moiety (Fig. 1B), have more contribution to their interaction energies with mTOR than other regions of these molecules. Altogether, the results of this work contribute to the understanding of the mTOR binding to ATP site inhibitors.

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TARGETING TOXIN-ANTITOXIN SYSTEMS WITH NEW RNA LIGANDS IN BACTERIA: TOWARDS NEW ANTIBIOTIC THERAPIES

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The discovery of new antibiotics is an important challenge in current medicinal chemistry. Due to multidrug resistance to current antimicrobial therapies[1], it is urgent to discover new targets for innovative antibiotic strategies and toxin-antitoxin (TA) systems represent promising targets for the development of innovative therapies.

The first examples of TA systems were identified in bacterial plasmids in the late ′80s, their implication in bacterial persistence, resistance to antibiotics as well as survival to various stress is becoming increasingly evident. TA systems are small genetic elements composed of a toxin gene and its related antitoxin both coding for corresponding toxin and antitoxin products. The toxins of all known TA systems are proteins that are either able to inhibit bacterial cell growth or to lead to cell death, whereas the antitoxins are proteins or small regulatory RNAs that neutralize the toxin. Here, we decided to target a type I TA systems where the antitoxin is a non-coding RNA that binds to the messenger RNA (mRNA) coding for the toxin thus inhibiting its translation [2]. For these reasons, we propose a series of new RNA ligands designed to inhibit the formation of toxin mRNA-antitoxin RNA complex thus leading to toxin production and bacterial death (Figure 1).

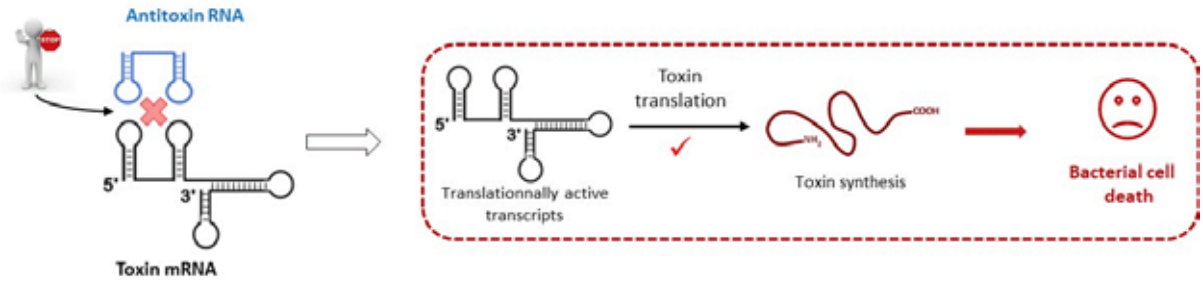

Figure 1. The inhibition of the interaction between toxin mRNA and the antitoxin RNA leads to a transcriptionally active mRNA, to toxin production and bacterial death.

In order to validate the proposed strategy, we target a particular type I TA system of the major human gastric pathogen *Helicobacter pylori* which is a gram-negative bacterium infecting about 50% of the entire world population[3].

Based on the experience of Dr. Duca research group about the design and the synthesis of selective RNA ligands, a large library of new compounds was designed using a multicomponent synthetic methodology[4]. The ability of the new compounds to disrupt the loop-loop interaction between the antitoxin and the toxin mRNA using *in vitro* assays is under evaluation. The most active compounds will be finally optimized in order to improve their biological activity and their pharmacodynamic/pharmacokinetic properties for a future therapeutic application. In conclusion, we present here an original approach toward the discovery of new antibacterial compounds against *H. pylori* infections bearing a completely new mechanism of action.

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L-PROLINE RELATED HETEROCYCLIC AMINO ACIDS AS BUILDING BLOCKS FOR SYNTHESIS OF 2-AMINO-1,3-SELENAZOLE-5-CARBOXYLATES

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(*S*)-Pyrrolidine-2-carboxylic acid, also known as L-proline, is a proteinogenic amino acid. It is an important building block for the preparation of various functionalized heterocyclic systems. When both the amino and carboxylic functional groups of proline are employed in the construction of a molecular skeleton, the formation of a chiral condensed heterocyclic system can be achieved.

Functionalized 1,3-selenazole moieties are present in many pharmacologically active compounds. Prominent examples include potent antiviral agent selenazofurin and histamine H2-receptor agonist amselamine. Recently, a series of novel 1,3-selenazole-containing 1,3,4-thiadiazole derivatives have been prepared, and their anticancer activity have been evaluated.

In the present work a series of methyl 2-amino-1,3-selenazole-5-carboxylates possessing a chiral pyrrolidin-2-yl, piperidin-2-yl or piperidin-3-yl substituent at C-4 of the heteroaromatic ring were designed and synthesized. The structures of the novel heterocyclic compounds were confirmed by ^{1}H, ^{13}C and ^{77}Se NMR spectroscopy and HRMS investigations.

The obtained chiral amino acids can potentially be used as scaffolds for the synthesis of more complex molecules or be used as building blocks for the development of DNA-encoded chemical libraries where peptide bond formation is necessary.

WATER EXTRACT OF CAPER BUSH LEAVES ENHANCES PHAGOCYTOSIS OF RAT NEUTROPHILS

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Plant flavonoids exhibit multiple biological activities. Many of them can behave as immune response modulators. Aim of this study was to evaluate the effect of flavonoid-rich extracts of caper bush (Capparis spinosa L.) on phagocytosis of rat blood neutrophils.

We used wild caper bush harvested near Homs (Syria) in 2018 and 2019. Leaves, stems or roots were extracted by water or 20% dimethyl sulfoxide (DMSO). The total phenolic content in extracts was determined using the Folin–Ciocalteu method and the total flavonoid content by $AlCl_3$ colorimetric method. The reducing capacity was assessed by the CUPRAC assay and the antioxidant activity by the ABTS decolourization assay. The cytotoxicity of extracts was evaluated in HeLa and Vero cell lines using the neutral red uptake assay. The phagocytic activity of rat blood neutrophils (intact and *E. coli* lipopolysaccharide (LPS)-stimulated) was examined against *Saccharomyces cerevisiae* cells. Based on the results of this assay percentage of activated neutrophils (PAN) and the phagocytic index (PhI) were calculated. The statistical analysis was performed by the Friedman test with the calculation of a post-hoc p-value by the Conover method with corrections by the Benjaminyi-Hochberg FDR method. The effects were interpreted as statistically significant at a p-value<0.05.

We found that in caper bush plants, the leaves have the highest phenol and flavanol contents. The phenol content in the leaves is 3-6 times higher than that in the stems and 3-8 times higher than in the roots. The flavonoid content in the leaves is 1.7-3.4 times higher than that in the stems and 1.6-4.3-times higher than in the roots. Flavonoids and total phenols from the leaves were better extracted by water than by 20% DMSO. Flavonoid concentrations in water extracts were in the range of 0.727-1.241 mM compared to 0.536-0.758 mM in DMSO extracts. Total phenol concentrations were 3.467-7.042 mM in water extracts and 1.639-3.732 mM in DMSO extracts. We observed a higher antioxidant activity and reducing capacity in water extracts in comparison with DMSO extracts which correlates with the current opinion that phenolic substances are major antioxidant and reducing agents in plants.

We tested the water and DMSO extracts of caper bush leaves for cytotoxic activity. DMSO extract exhibited concentration-dependent cytotoxicity in a non-transformed mammalian cell line (Vero). In contrast, DMSO extract had no cytotoxic effect in a tumour human cell line (HeLa). Water extract of caper bush leaves had no influence on cell viability in both tested cell lines (Vero and HeLa). Based on these results, we tested only the effects of water extract of caper bush leaves on phagocytosis of rat neutrophils.

The water extract of caper bush leaves at concentrations of at 1 μM of total phenols significantly stimulated basal phagocytic activity (PAN +38.1% and PhI +17.0%) without any significant effects on LPS-stimulated phagocytosis. Known phagocytosis modulators (1 μM gallic acid and 1 μM epigallocatechin 3-O-gallate (EGCG)) were used as positive controls. Gallic acid markedly enhanced basal phagocytic activity (PAN +29.8% and PhI +11.2%) and exhibited no significant effects on LPS-stimulated phagocytosis. EGCG stimulated basal phagocytic activity (PAN +9.5% and PhI +12.8%) and decreased PAN (-22.4%) in case of LPS-stimulated phagocytosis without influence on PhI.

Therefore, we found that flavonoid-rich water extract of caper bush leaves can enhance basal (but not LPS-stimulated) phagocytic activity of rat blood neutrophils at comparable levels and in a manner similar to known phagocytosis modulator, gallic acid.

SapL1: A NEW TARGET LECTIN FOR THE DEVELOPMENT OF ANTIADHESIVE THERAPY AGAINST Scedosporium apiospermum

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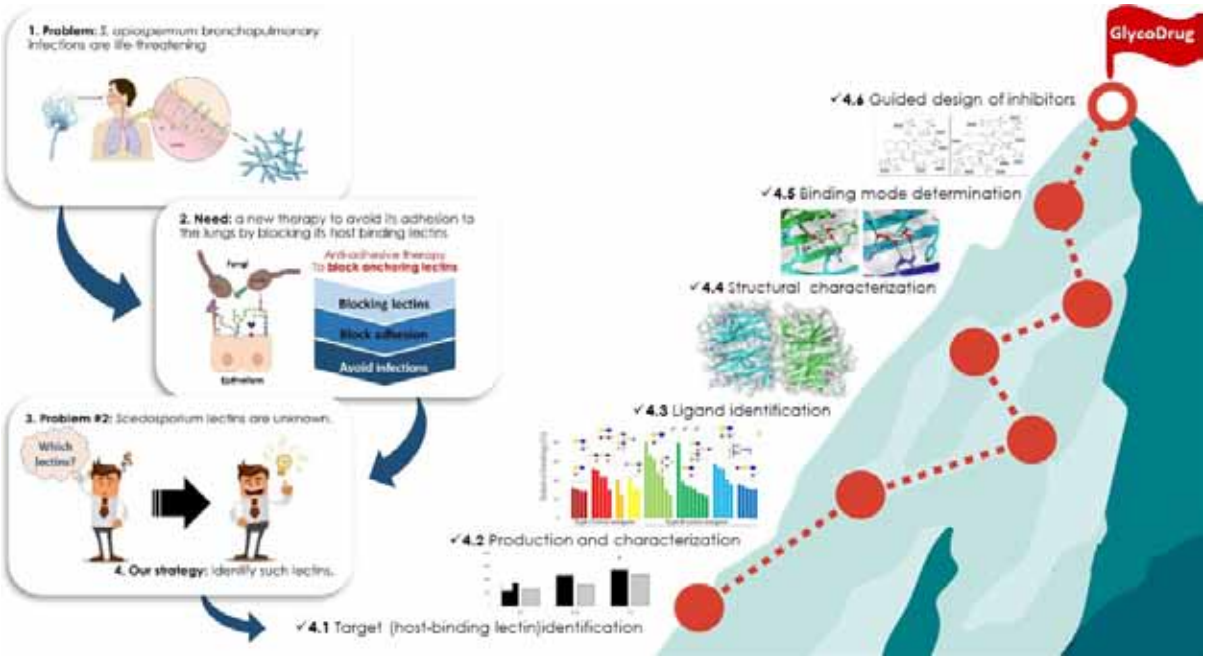
Scedosporium apiospermum is an emerging opportunistic fungal pathogen responsible for life-threatening infections in immunocompromised patients. It represents a therapeutic challenge due to its intrinsic resistance towards antifungals and to its high rate of recidivism. Therefore, there is an urgent need to generate new therapies against this pathogen.

Anti-adhesive therapies have recently become very important in the treatment of opportunistic pathogens because they can be administrated prophylactically to immunocompromised patients. These therapies aim to avoid the first stage of infections by **blocking the proteins that are responsible for the anchoring of pathogens to the host-cells**. Consequently, they don't induce selective pressure that leads towards drug resistance generation. Unfortunately, due to its emerging character, ***Scedosporium* pathogeny and virulence factors are still unknown**, becoming the main bottleneck for anti-adhesive therapy development.

Here, we present the first report of identification and characterization of a *S. apiospermum* lectin (SapL1), its recombinant production, an analysis of its fine specificity and affinity by Glycan array and Isothermal Titration Calorimetry (ITC), as well as **the structural characterization of its binding mode by X–ray crystallography**.

SapL1 is homologous to a conidial surface lectin from *Aspergillus fumigatus* (FleA) which is involved in adhesion to host cells. It was identified by data mining using the FleA sequence as a bite into the *S. apiospermum* genome. According to the glycan array data, SapL1 is strictly specific for fucosylated carbohydrates and its refined model exhibits a dimer of six-bladed β-propellers with six binding sites found at the interface between blades. Binding sites are non-equivalent but they all share important features necessary for fucose recognition, such as triad of conserved residues involved in essential hydrogen bonds with the hydroxyls of C(2) and C(3) of the sugar, and hydrophobic interactions with the C(6). Conversely the C(2)OH seems to be the most versatile position, since it interact differently with a protein loop adjacent to four of the binding sites. Those pockets display higher affinity for the ligand, indicating that such interactions are responsible for enhancing the affinity and might be explored for design of potent inhibitors. This information contributes to the understanding of human glycosylated surfaces recognition by *Scedosporium,* and now is **leading the development of glycodrugs** for antiadhesive therapies against this pathogen by targeting SapL1 inhibition.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 765581.

NEW BENZIMIDAZOLE ARYLHYDRAZONE HYBRIDS AS POTENTIAL MULTI-TARGET DRUGS FOR THE TREATMENT OF PARKINSON'S DISEASE

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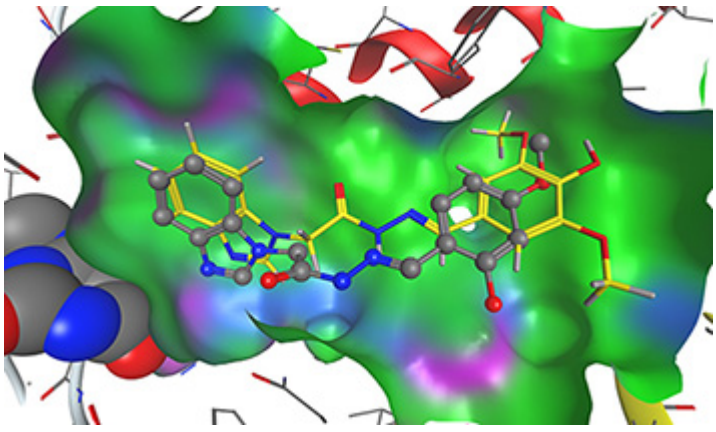
Parkinson's disease (PD) is one of the most prevalent and still incurable neurodegenerative disorders characterized by progressive death of dopaminergic neurons. Neuroprotective drugs and selective monoamine oxidase inhibitors can slow down the progression and improve symptoms. There is an implication of oxidative stress in the decease pathophysiological mechanisms, making compounds possessing the ability to reduce oxidative stress the prime candidates for neuroprotection. Thereby our current study is focused on the development of new PD drugs with combined MAO-B inhibiting, neuroprotective and antioxidant properties. A small series of benzimidazole derivatives containing hydroxy and methoxy arylhydrazone fragments has been synthesized and the neurotoxicity of the compounds has been evaluated in vitro on neuroblastoma SH-SY5Y cells and on isolated rat brain synaptosomes measuring the cell viability and levels of reduced GSH, showing a good safety profile as compounds 7 was the least toxic. The neuroprotective properties of the compounds were assessed also on two models: H2O2 induced oxidative stress on SH-SY5Y cells where compounds 7 showed more pronounced neuroprotective activity than the referent melatonin and rasagiline, and on 6-OHDA induced neurotoxicity in rat brain synaptosomes where the neuroprotective capability of 7 to preserve the synaptosomal viability and the GSH levels exceeded the one rasagiline and was similar to the one of melatonin. The compounds were tested in inhibiting the activity of human MAO-B where all compounds showed significant inhibition but once again 7 exerted the most prominent activity similar to those of selegiline and rasagiline. The carried molecular docking studies revealed that its activity is related to its appropriate molecular structure enabling the ligand to enter deeper in the narrow and highly lipophylic active site pocket of the hMAO-B and has a favouring interaction with the key amino acid residues Tyr326 and Cys172. As much scientific evidence points out the implication of iron dyshomeostasis in PD, the compounds were tested to reduce the ferrous iron induced oxidative molecular damage on biologically important molecules was tested in vitro in a lecithin containing model system. All investigated compounds denoted protection effect, stronger than the one of the referent melatonin. In order to support the assignments of the significant neuroprotective and antioxidant pharmacological activities, the radical-scavenging mechanisms of the most promising compound 7 were evaluated using DFT methods. In the present current study outlines a perspective leading structure, bearing the potential for a new anti-PD drug.



MULTIDRUG RESISTANCE MARKS THIS DECADE! ANTIBIOTIC ADJUVANTS TO RESCUE PSEUDOMONAS AERUGINOSA FROM RESISTANCE

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Multidrug resistant (MDR) bacteria are one of the major current threats to public health. The incidence of nosocomial infections caused by MDR has increased dramatically in the community and is associated with a terrifying rate of morbidity, mortality and antibiotic use.

The molecular mechanisms by which bacteria, particularly Gram-negatives, became resistant to antibiotics are diverse and complex. Nowadays, Bacteria have developed resistance to all different classes of antibiotics discovered.

Without an innovative strategy to combat multi-drug resistant (MDR) pathogens, many fields of medicine will be severely affected, thus, new solutions are required to enhance the antibiotic efficiency and/or reduce the mechanism of resistance.

In this context, we aim to develop an attractive strategy consisting in the synthesis of new polyaminisoprenyl molecules. These chemo-sensitizers are readily prepared from farnesol in a two steps synthesis with moderate to excellent yields and they are able to restore the activity of doxycycline against *pseudomonas aeruginosa* bacterial strains.

We demonstrate that among 7 analogues tested one of them presented an excellent capacity to enhance doxycycline activity. Typically, this compound named NV716 was assayed against Gram negative strains and proved to act in a synergetic manner with doxycycline (2mg/L) at very low concentrations in the range of 2.5-10 µM. Thus we showed that NV716 is able to decrease the MIC of doxycycline on various reference strains of *pseudomonas aeruginosa* to the sensibility level (2mg/L). The mechanism of action of this compound is now under current investigation.

THE FIRST BERBERINE-BASED INHIBITORS OF TYROSYL-DNA PHOSPHODIESTERASE 1 (TDP1)

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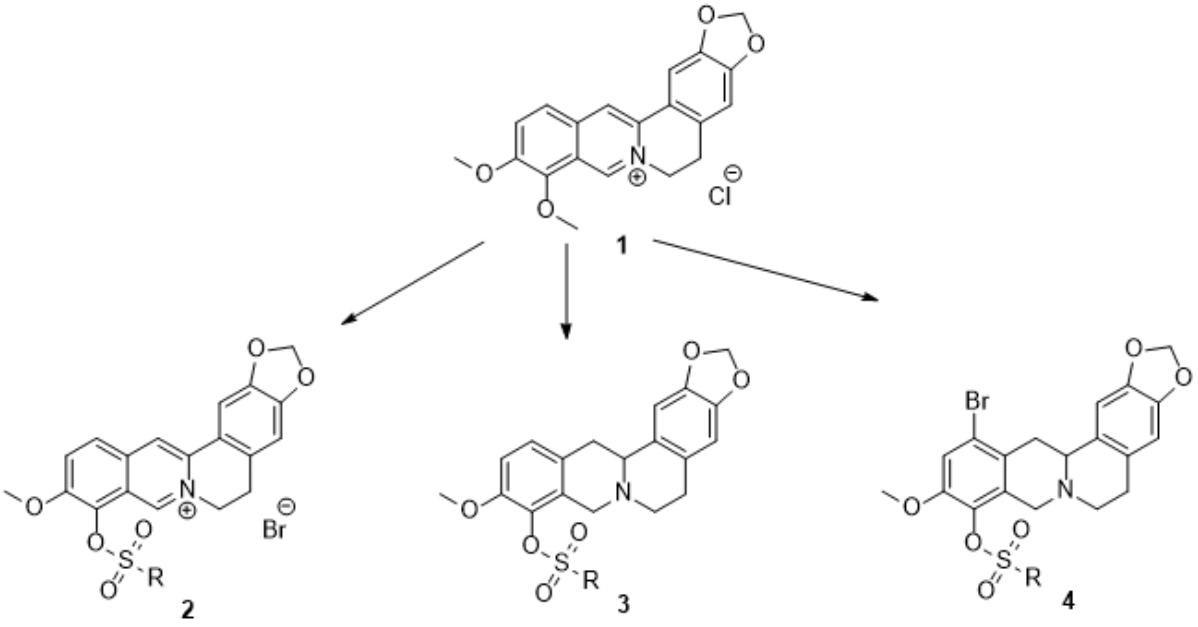
A promising strategy to enhance the efficacy of anticancer therapy includes the inhibition of various DNA repair enzymes, which counteract the effect of many anticancer drugs [1]. The berberine and tetrahydroberberine sulfonates and their brominated analogues **2-4** have been synthesized and tested to evaluate their tyrosyl-DNA phosphodiesterase 1 (Tdp1) inhibitory activity. As far as we know, for the first time it was shown that berberine-based compounds have potential as inhibitors of this enzyme. The IC_{50} values were in the range from 0.53 μM to 4 μM. Among the alkyl sulfonates, only derivatives with bromine substitution at site 12 of tetrahydroberberrubine were active in the micromolar range. At the same time, both tetrahydroberberrubine and 12-bromtetrahydroberberrubine derivatives containing polyfluoroaromatic substituents all have inhibitory activity with IC_{50} values of ~1 μM. According to the inhibitory activity, toxicity data and ability to sensitize topotecan against HeLa cervical cancer cell line the two most promising derivatives **3g** and **4g** were identified. These compounds doubled the cytotoxic effect of topotecan at concentrations of 5-20 μM.



2a: R = CH_3;
2b: R = C_2H_5;
2c: R = C_3H_7;
2d: R = C_4H_9

3a: R = CH_3;
3b: R = C_2H_5;
3c: R = C_3H_7;
3d: R = C_4H_9;
3e: R = C_6F_5;
3f: R = p-C_6F_4H;
3g: R = p-$C_6F_4CF_3$

4a: R = CH_3;
4b: R = C_2H_5;
4c: R = C_3H_7;
4d: R = C_4H_9;
4e: R = C_6F_5;
4f: R = p-C_6F_4H;
4g: R = p-$C_6F_4CF_3$

These results indicate that sulfonates of tetrahydroberberine have potential to be developed as new agents for anticancer therapy due to their promising Tdp1 inhibitory activity.

This work was supported by Russian Science Foundation (grant 19-13-00040)

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NOVEL BODIPY DYES: DESIGN, SYNTHESIS AND ASSESSMENT OF THEIR PHOTOPHYSICAL PROPERTIES

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Fluorescent dyes have emerged as a powerful tool for visualizing and labelling biomolecules *in vitro* and *in vivo*. Boron-dipyrromethenes (BODIPYs) play a pivotal role in fluorescent probe development due to their favorable physicochemical properties, their ease of synthesis and the wide range of applications in various research fields. [1,2]

Series of new mono-, di- and tri-substituted bodipy derivatives incorporating heterocyclic groups were synthesized. Starting from 1,3,5,7-tetramethyl-8-phenyl-4,4-difluoroboradiazaindacene the synthesis of the new dyes was effected through a sequence of condensations and cross-couplings reactions. The effect of the new substituents on the photochemical and photophysical properties of the new dyes was investigated. The spectroscopic properties of the new dyes were measured in different solvents while, the pH stability was assessed in various buffer solutions.

Initially, we embarked on the synthesis of the mono-substituted analogues through a Knoevenangel condensation reaction of the corresponding aldehydes either at the α or at the β site of 1,3,5,7-tetramethyl-8-phenyl-4,4-difluoro-boradiazaindacene. Subsequently, we extended the π-conjugation system of the bodipy core, through the synthesis of di- and tri-substituted derivatives, in order to generate red-shifted bodipy-dyes with improved optical properties. Specifically, the new derivatives were generated through a Vilsmeier-Haack reaction at C2 or C3 to generate the different heterocyclic scaffolds and derivatization through Cross-coupling reactions (Suzuki and Sonogashira) at C6. Conversely, the 2,3,5-trisubstituted derivatives were obtained through a condensation reaction of the C3 and C5 methyl groups with aromatic aldehydes under microwave irradiation and derivatization at C2 (Condensation or cross-coupling reactions).

In conclusion, we recently designed and synthesized new fluorescent bodipy-dyes, bearing heterocyclic substituents at different positions of the bodipy scaffold. The new compounds exhibited a bathochromic shift compared to that of 1,3,5,7-tetramethyl-8-phenyl-4,4-difluoroboradiazaindacene. Studies involving further optimization of the optical properties of the new dyes, DFT-calculations and *in vitro* studies on their potential bio-applications are currently ongoing.

Acknowledgement

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INHIBITION OF QUORUM SENSING WITH NOVEL CHLOROQUINE FUMARDIAMIDES

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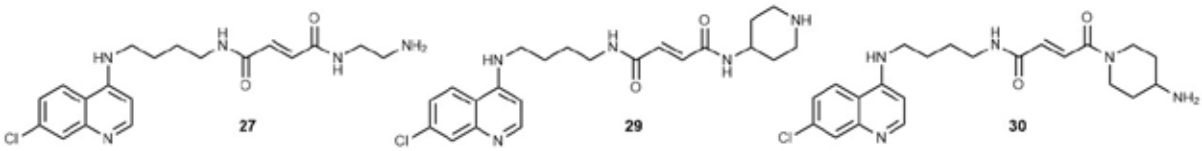
Most microbial pathogens exploit adherent growth on various surfaces (i.e., biofilm formation) in order to avoid the effect of antibiotics and host immune cells. A cell-to-cell communication, termed quorum sensing (QS), plays a key role in coordinating the biofilm formation and various other pathogenic pathways.[1] By utilizing QS, microbial cells can detect and respond to cell population density via the production and release of a chemical signal molecule called autoinducer. It is considered that molecules or compounds able to interrupt QS pose less selective pressure on the microbes than the conventional antibiotics, thus, contributing less to antibiotic resistance.[2] Strategies that interrupt the QS-communication outside the cell, also called quorum quenching (QQ), are highly promising since the communication system can be blocked without entering the cell. Therefore, search for novel anti-QS compounds preventing the development of specific group behavior and causing chemical attenuation of biofilm formation is considered the method of the choice to combat biofilm infections without boosting the resistance.[3] Quinoline derivatives were shown as effective antagonists of PqsR, the receptor of pqs system of the human pathogen *Pseudomonas aeruginosa*, which controls the expression of various virulence factors and is involved in the biofilm formation.[4]

We report here the synthesis and activity of four novel fumardiamides with chloroquine (CQ) moiety on one side and scaffolds bearing primary or secondary amino groups on the opposite side of the molecule. The *C. violaceum* ATCC31532 and CV026 strains were used as QS reporters for testing anti-QS and bactericidal activities. Our results revealed that compounds **27**, **29** and **30** inhibited QS by approx. 46%, which is similar to the activity of quercetin, the positive control for QS (56%).The cell viability staining indicated the tested compounds showed a negligible bactericidal effect in both strains ranging from 6.5 to 16.2%. Since the compounds had no bactericidal effect on the *C. violaceum* cells, we considered them as new QQs able to block QS without inducing natural selection pressure as traditional antimicrobial agents. Our results could help in further design of agents targeting cell-to-cell communication and may open new avenues for the combat against drug-resistant bacteria.



The study was supported by the Croatian Science Foundation through the research project IP-09-2014-1501 and the Jane and Aatos Erkko Foundation (Helsinki, Finland). The work of doctoral student M. Beus has been fully supported by the Young researcher's career development project – training of doctoral students of the Croatian Science Foundation founded by the European Union from the European Social Fund.

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DESIGN AND SCREENING OF A PEPTIDOMIMETIC DNA-ENCODED LIBRARY - IDENTIFICATION OF TEAD-YAP-INHIBITORS

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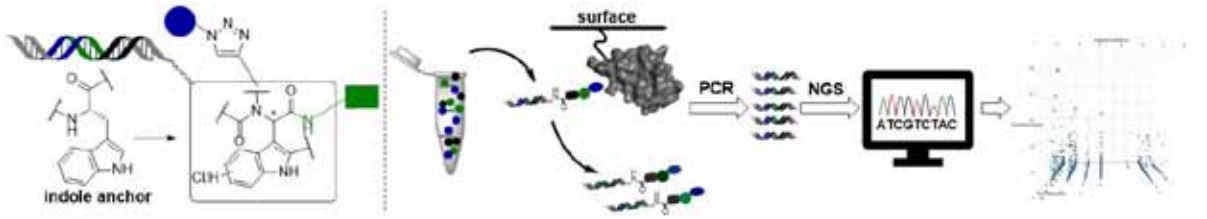
DNA-encoded libraries (DELs) offer a high-throughput platform for screening of chemically synthesized small molecules.[1] They are synthesized by DNA-encoded combinatorial chemistry. One important challenge in DEL technology is the development of a comprehensive toolbox of reactions for diverse library design. Due to their robustness and scope, isocyanide multicomponent reactions (MCRs) play a prominent role in drug discovery.[2] We established the compatibility of four different isocyanide MCRs with our DNA barcoding strategies.[2] Moreover, we demonstrate the synthesis of an indole-focused peptidomimetic DEL initiated with a chemically stable thymidine adapter oligonucleotide ("tiDEL").[3,4] The peptidomimetic backbone was synthesized by the Ugi multicomponent reaction. tiDEL synthesis was continued with DNA barcode ligation, and azide-alkyne cycloaddition in the combinatorial step. We aimed at identifying inhibitors for human TEAD4 (hTEAD4), a tumor-associated transcription factor and effector of Hippo signaling. Selection of the tiDEL on hTEAD4, next generation sequencing (NGS) and analysis of the sequencing data with a newly developed algorithm indicated a class of compounds binding to the target protein. An exemplary compound disrupted the hTEAD4-YAP interaction *in vitro* and reduced the expression of a gene under the control of the TEAD-YAP transcription factor complex. It may serve as starting point for development of a novel class of anticancer agents.



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DISCOVERY OF ARG1 INHIBITORS DESIGN AND SYNTHESIS OF BORONIC SUBSTITUTED HETEROCYCLES

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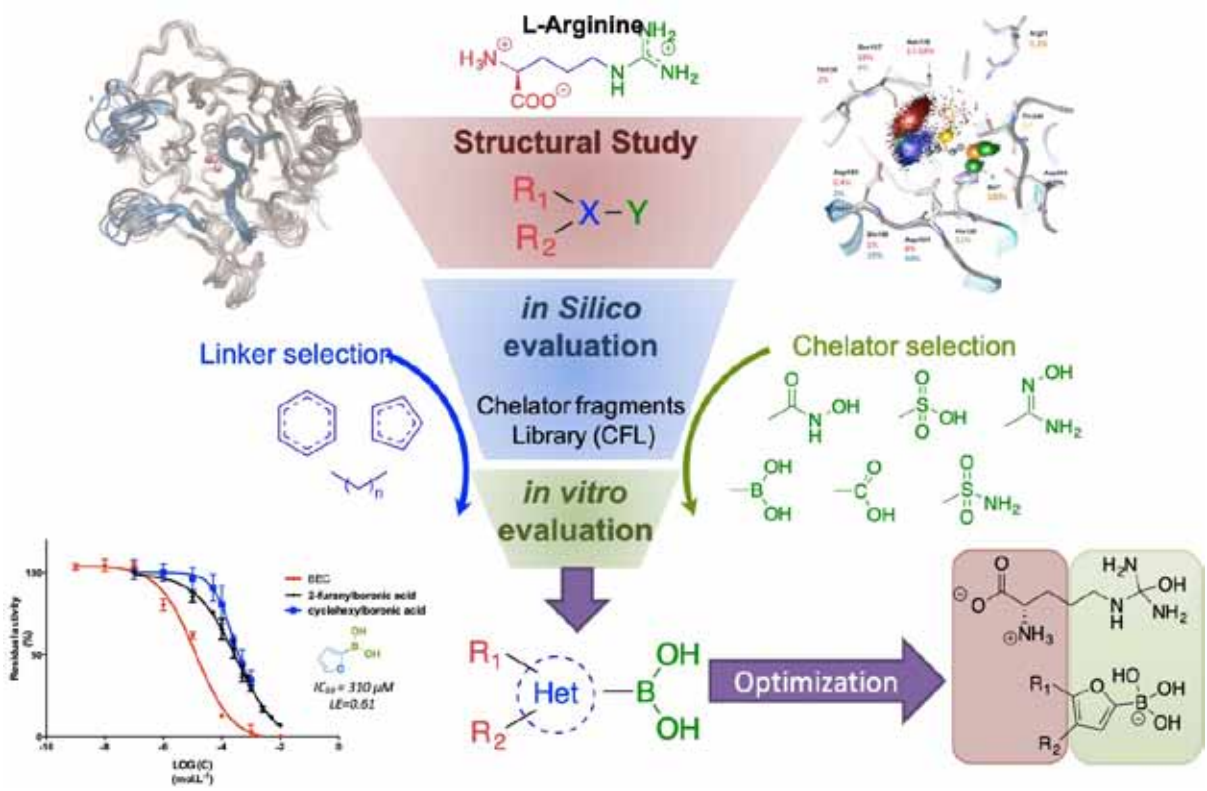
Despite major advances in understanding the mechanisms leading to tumor immunity, the discovery of effective therapies is confronted to numerous difficulties. Such obstacles include the ability of tumors to foster a tolerant microenvironment and the activation of a plethora of immunosuppressive mechanisms. Among others major mechanisms, immunosuppression involves the catabolism of L-tryptophan and L-Arginine. In the context of L-arginine depletion, Arginase 1 (ARG1) is a therapeutically relevant target in tumor tolerance, synergistically associated with tumor growth.



Our current strategy leading to better understanding of molecular mechanism of ARG1 will be presented as well as our advancements in the discovery of inhibitors to use as pharmacological tools. A rational structure based drug design was combined with a in depth computer-aided investigation, including molecular dynamics and construction of dynamic 3D pharmacophore (dynophores). Building up Chelator Fragments Library (CFL) as innovative approach specifically targeting metals of ARG1 highlighted the interest of study the linker moiety. Assessment of cycle and heterocycle derivatives was confirmed by experimental testing with a targeted fragment library focused on the more efficient chelating function, *i.e.* boronic acid.

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SYNTHESIS AND ANALGESIC ACTIVITY OF NEW BENZO[1,3]OXAZINES BASED ON MONOTERPENOID (-)-ISOPULEGOL

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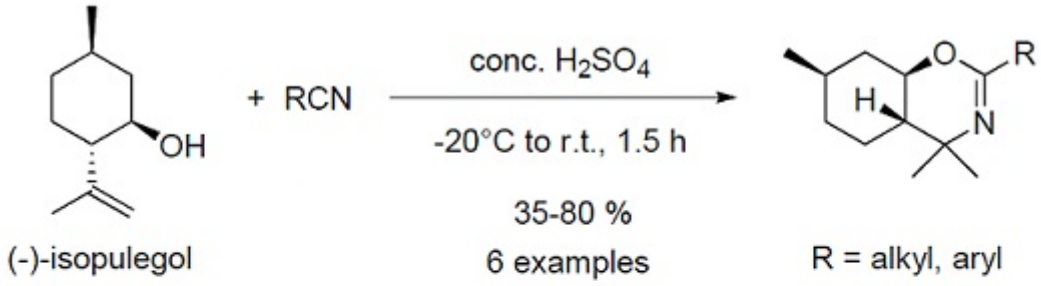
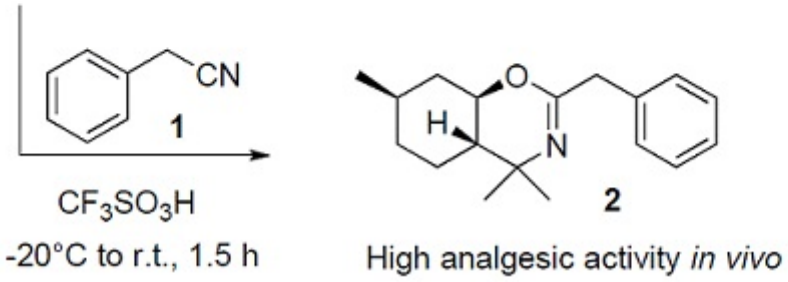
Oxazines, heterocyclic compounds containing one nitrogen and one oxygen atom in a six-membered ring, have been of great interest to medicinal chemists in recent decades [1]. This is especially true for compounds containing the 1,3-oxazine moiety due to the wide range of biological activity they exhibit, including anticancer, antibacterial, antifungal and etc.

We found that the Ritter reaction between monoterpenoid (–)-isopulegol and a number of aliphatic and aromatic nitriles in the presence of concentrated sulfuric acid led to a series of chiral 1,3-oxazine derivatives with 35–80 % yields. Carrying out the reaction of (–)-isopulegol with benzyl cyanide **1** in the presence of CF_3SO_3H made it possible to increase the yield of the target product **2** from 40 %, achieved in the presence of H_2SO_4, to 60 %.





The search for new analgesic agents with high activity and low toxicity is of primary importance. It is known that some heterocyclic compounds synthesized from (–)-isopulegol exhibit analgesic activity [2]. When studying the analgesic activity of the synthesized compounds *in vivo*, it was found that 1,3-oxazine **2** at a dose of 10 mg/kg exhibited significant analgesic activity reliably increasing the latent time of animals being on the hot plate and not inferior in efficiency to the reference drug diclofenac sodium administered at a similar dose.

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BIOMIMETICS AND BIOHYBRID STRUCTURES AS TOOLS FOR CONTROLLING MOLECULAR STRUCTURE

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Lytic polysaccharide monooxygenases (LPMO) are copper-containing metalloenzymes that oxidatively cleave the glycosidic linkages in polysaccharides via C-H oxidation.1 The active site of LPMO contains a single copper atom in a T-shaped N_3 coordination environment known as the histidine brace. This structural motif is conserved across all members of LPMO and yet its role in the oxidative power of these enzymes is still not well understood. In part, this results from the lack of synthetic model systems that can replicate this first coordination sphere, allowing detailed study of this ligand set.

The problem here is that typical ligands for coordination chemistry allow the metal to control a large part of the coordination geometry. A system that could provide a structurally stable pre-organized binding site would greatly advance the design of biomimetic models. This poster describes our approach towards the use of folded organic scaffolds for generating pre-organized binding sites for metal complexes. Through the functionalization of an oligoamide scaffold with imidazole and histamine groups, the histidine brace motif, including the trans imidazoles and primary amine, can be formed as a results from the folding preferences of the aromatic oligoamide chain. Moreover, additional functionalization of the scaffold can allow second coordination sphere interactions to be included, giving the opportunity to delineate the precise role of the histidine brace on the metal based reactivity. The initial synthetic progress and the perspectives for this project are described.

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DESIGN AND BIOPHYSICAL CHARACTERIZATION OF FATTY ACID METABOLISM INHIBITORS CONJUGATED TO PH-SELECTIVE CANCER CELL DELIVERY TOOLS

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The pH Low Insertion Peptides (pHLIP) family, derived from the bacteriorhodopsin C helix, represents a unique class of water-soluble membrane polypeptides able to insert across a membrane, forming a stable transmembrane α-helix[1,2]. Under neutral and high pH conditions, the pHLIP is soluble in the presence of a lipid bilayer; it is monomeric and binds to the membrane in a random coil conformation. In an acidic environment, the transmembrane part (TM) of the peptide spontaneously folds into an α-helical conformation, gets inserted into the membrane, crossing the lipid bilayer.

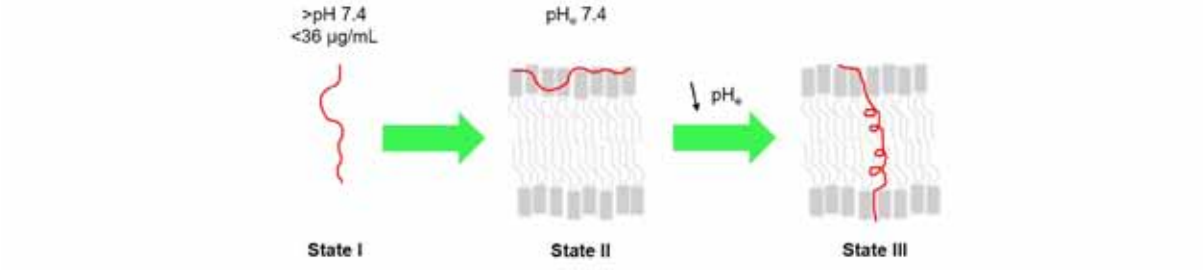

Figure 1: Representation of the three states of a pHLIP

Contrary to healthy tissues, tumors often exhibit a pH gradient with an acidic extracellular space and slightly alkaline cytoplasm. In the most acidic tumor compartments, cancer cells adapt their metabolism by different processes. Notably, acidic cancer cells present an increase in fatty acid uptake and accumulate them as triglycerides into lipid droplets (LDs) [3]. These energy reserves are used by invading cancer cells upon fatty acid oxidation (FAO) as a source of energy to resist anoikis[3]. Inhibitors of FAO and LD formation have therefore the potential to block the acidosis-supported invasive potential of cancer cells. Since enzymes involved in fatty acid metabolism are shared by some healthy tissues, coupling the above inhibitors to a pHLIP would allow the selective targeting of tumor cells.

The goal of this work is to establish the pHLIP efficacy in the selective delivery of cargo molecules to the acidic tumor microenvironment. As such, inhibitors were selected, synthesized, and chemically modified for conjugation. Based on an in-depth literature study of potential peptides, we designed a new pHLIP peptide. Then, we coupled several modified inhibitors with the designed pHLIP (pHLIP*d*) and with a reference pHLIP (pHLIP*r*).The conjugates were purified and characterized by HPLC-MS. pHLIP*d* and the conjugates were evaluated using intrinsic tryptophan fluorescence and circular dichroism (CD) in presence and in absence of liposomes as a model of lipid bilayer at pH 4 and pH 8. Further, we are currently evaluating of the conjugates on 3D tumor spheroids wherein acidosis spontaneously develop.

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NOVEL NON-BASIC DOPAMINE D2 RECEPTOR ANTAGONIST (D2AAK2) AS A POTENTIAL ANTIPSYCHOTIC – STUDIES ON ITS OPTIMIZATION AND THE BINDING MODE TO D2 RECEPTOR

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The effectiveness of the treatment of schizophrenia is still limited due to complexity of the disease and the fact that antipsychotics available on the market often fail in reducing negative or cognitive symptoms. In previously conducted structure-based virtual screening aimed at identifying novel dopamine D_2 receptor antagonists, compound D2AAK2 was found [1]. *In silico*, *in vitro* and *in vivo* evaluation of the lead structure revealed its affinity to key molecular targets engaged in the pathomechanism of schizophrenia, as well as its antipsychotic, anxiogenic and procognitive effects. Interestingly, the compound does not possess a protonatable nitrogen atom which constitutes the main element of interaction with orthosteric binding site of aminergic G protein-coupled receptors. However, it possesses an amide group with a hydrogen atom, that may possibly interact with the conserved Asp(3.32) of dopamine D_2 receptor. In order to optimize the lead structure and to study its binding mode, a series of its analogues was synthesized. Surprisingly none of the derivatives showed affinity for D_2 receptor in *in vitro* radioligand binding assays. In one of the analogues hydrogen atom of the amide group was replaced with methyl substituent, resulting in loss of activity. This supports the hypothesis of the orthosteric binding mode. However, three other obtained derivatives with additional basic nitrogen atom also turned out to be inactive which is in opposition to orthosteric mode of action and may suggest that D2AAK2 is the allosteric modulator of dopamine D_2 receptor. Further studies are required to discover the way D2AAK2 interacts with its molecular target.

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NEUROPROTECTIVE CELLULAR AND MOLECULAR MECHANISMS IN NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases are one of the greatest challenges for science and clinical medicine due to their complex pathology and the lack of treatment methods based on mechanisms [1]. Emerging neuronal dysfunction is associated with cellular and molecular changes that trigger a cascade of events and, consequently, the death of nerve cells. The changes involved in neurodegeneration include an increased level of oxidative stress, impairment of mitochondrial function, activation of apoptotic factors, an abnormal mechanism of the cell cycle as well as an increased level of cellular calcium and DNA damage. Currently, research on pharmacological compounds focuses on halted or reversing changes that have occurred in the brain, while minimizing side effects. However, an effective drug therapy for treating neurodegenerative diseases has not yet been developed [2].

The presented research focuses on developing a potential effective treatment with neuroprotective effects based on basic cellular and molecular changes.

A multi-target antipsychotic compound was used in the conducted research [3] and two cell lines: mouse hippocampal neuron (HT-22) and human neuroblastoma line (SH-SY5Y). Tests were performed to evaluate the production of reactive oxygen and nitrogen species (ROS and RNS, respectively) and on the activation of mechanisms to combat free radicals (reduction of glutathione). We also show tests for cellular calcium, cell cycle and DNA damage in the form of micronuclei.

Summarizing, the obtained results represent a promising starting point to the development of treatment for neurodegenerative disorders based on cellular mechanisms.

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OPEN SCIENCE MEDICINAL CHEMISTRY: TOWARDS A TREATMENT FOR DIPG

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Meds4Kids Pharma (M4K)[1] is pioneering an open science approach to drug discovery, focussed on the discovery and development of small molecule therapeutics for orphan paediatric cancers. M4K is testing the hypothesis that an open science framework can be successfully applied not only to accelerate basic science, using the collective knowledge of the scientific community at large, but also to take an innovative new drug candidate all the way from discovery and clinical proof-of-concept through to product registration, by making use of regulatory data protections and market incentives. Since late 2017, Charles River Early Discovery has been providing in kind drug discovery services to help progress these efforts, including medicinal and synthetic chemistry.

Diffuse intrinsic pontine glioma (DIPG) is a rare, aggressive and uniformly fatal childhood brain cancer with a median survival time of 9-12 months and for which there are currently no effective drug treatments. The disease has been shown to be associated with mutations in the ACVR1 gene (activin A receptor, type 1) also known as ALK2 kinase. Early support for the therapeutic hypothesis that an inhibitor of ALK2 kinase would have clinical benefit in DIPG, came from in vivo studies with non-selective ALK2 kinase inhibitors, which both killed DIPG cell lines harbouring the ALK2 mutation and extended lifespan in xenograft mouse models.[2]

The open science approach has been utilised to develop a series of potent, selective, orally bioavailable and brain penetrant inhibitors of ALK2 based on the lead compound LDN-214117. Modifications to the core pyridine substituents gave three leading compounds, each with superior potency, selectivity and / or brain penetration profiles. Further PK and tolerability studies confirmed the interest of these compounds as potential candidates for evaluation in mouse models of DIPG.

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REDUCTION OF 2,4,5-TRIARYLIMIDAZOLINES AS A METHOD FOR THE PREPARATION OF VICINAL DIAMINES AND PYRAZINES

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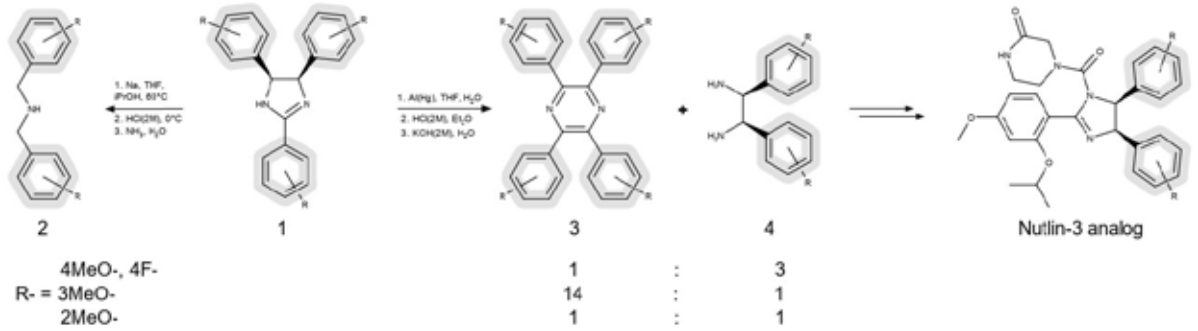
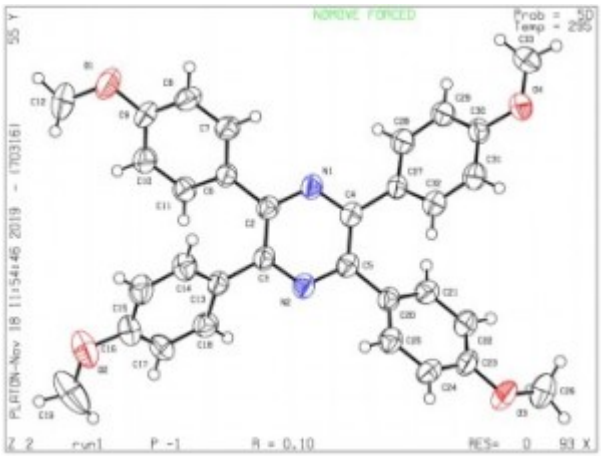
Vicinal diarylethylenediamines are widely used in enantioselective organic synthesis as chiral catalysts and as precursors in the production of nutlins, compounds used in cancer therapy. A convenient way to obtain cis-diamines is the reduction of the corresponding substituted imidazolines which are easily produced from aldehydes and ammonia [1]. It was shown for the first time that the reduction of imidazolines using aluminum amalgam, depending on the reaction conditions, can be obtained both diamines and 2,3,5,6-tetraarylpyrazines. Tetraarylpyrazines may be used as regulators of blood glucose level and may also have anti-tumor activity.

We investigated two methods of the imidazolines reduction (1). In the first method metallic sodium was used as the reducing agent and N,N-dibenzylamines (2) were obtained. In the second method aluminum amalgam was used and the mixture of 2,3,5,6-tetraarylpyrazines (3) and 1,2-diarylethylenediamines (4) was obtained. The ratio of the latter differs sharply for different substituents. The main product of the reaction with 4-substituted imidazoline is diamine. On the contrary in the reaction with 3-substituted imidazoline pyrazines are mainly obtained. 2-substituted imidazolines are barely converted.



The structure of tetraarylpyrazines was confirmed by single-crystal X-ray diffraction.



Acknowledgments: This work was supported by the Russian Foundation for Basic Research (Project 20-03-00915A)

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STRUCTURE-ACTIVITY STUDY AND MOLECULAR INSIGHTS INTO THE MODE OF ACTION OF THE COMPLEMENT C3 INHIBITOR CP40

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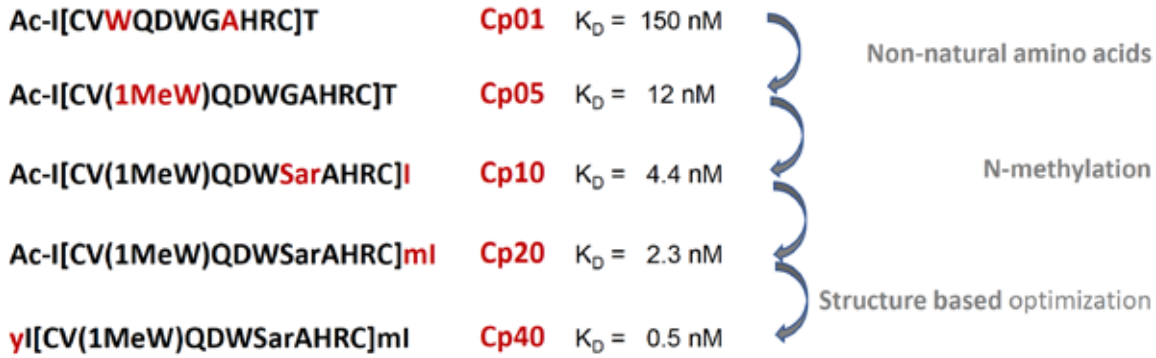
The complement system serves as “first line of defense” against injurious stimuli and invaders in blood circulation. Upon activation, a series of cascading enzymatic reactions lead to amplification of the response and to opsonic clearance and/or direct killing of the pathogen. Yet, complement has gained increasing interest as a potential drug target, since it may be inadvertently triggered and contribute to clinical complications in the pathogenesis of various autoimmune, inflammatory and age-related diseases as well as transplant rejection. While the involvement of dysregulated complement activation in inflammatory and autoimmune diseases is now widely recognized[1], so far only one class of complement-specific drugs has reached the market. These antibodies target the terminal step of the complement cascade, leaving the amplification loop and inflammatory signaling, mediated by complement component C3, active. The peptidic C3 inhibitor compstatin was originally identified by phage display[2] and optimized towards a picomolar cyclic peptide Cp40. Several of its derivatives have reached clinical development for the treatment of paroxysmal nocturnal hemoglobinuria (PNH), age-related macular degeneration (AMD) and periodontal disease, and a Cp40-based drug is currently being tested for efficacy in COVID-19-induced acute respiratory distress syndrome.



A detailed molecular understanding of the target binding profile and mode of action of this drug class is critical for clinical development and the design of future analogs. Here we aimed to identify key target interaction determinants of compstatin derivative Cp40 by a structure-activity relationship study. By analyzing the co-crystal structure of Cp40 in complex with C3b, and performing computational simulations, we identified dTyr-1, (1Me)W-5, Gln-6, Trp-8, Sar-9, Ala-10, His-11, mIle-14 as key contact residues, and quantified their individual contributions to Cp40’s affinity. We thereby identified (1Me)W-5 and dTyr-1 as major determinants of target residence time. Interestingly, we also identified notable influence of amino acids that are not in direct contact with the target and were previously considered auxiliary rather than affinity-defining. Finally, we performed direct binding studies that confirmed and extended earlier hypotheses about compstatin’s mode of action.

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SYNTHESIS OF TRIMETHOXYCINNAMIC ACID ESTERS

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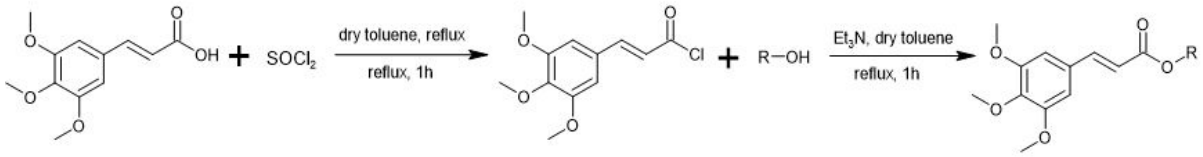
In the search for new pharmacologically active substances, cinnamic acid and its derivatives are important promising substances with great potential for the development of new drugs. In recent decades, cinnamic acid and its derivatives have attracted the attention of scientists due to low toxicity and a wide range of biological activities such as antibacterial, antiviral, anti-inflammatory, cytotoxic, antidiabetic, hepatoprotective, antioxidant, neuroprotective, anxiolytic, antituberculotic and antimalarial activity. The aim of this study is the preparation of trimethoxycinnamic acid esters. The synthesis consisted of two steps, in the first step acyl chloride of trimethoxycinnamic acid was prepared. In the second step acyl chloride reacted with alcohol. All prepared compounds were identified by using spectral methods (IR, ^{1}H-NMR, ^{13}C-NMR, MS) and sent for evaluation of the biological activity.



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APPLICATION OF (5-AMINO-2-PYRIDYL) 1-THIOGLYCOSIDES DERIVATIVES TO INHIBIT THE GROWTH OF COPPER IONS DEPENDENT CANCER CELLS

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Despite significant progress in the treatment of various types of diseases, cancer is still one of the most considerable clinical problems. Anticancer therapies introduced so far are not effective enough. This is due to the low selectivity profile of approved chemotherapeutics. Achieving the desired therapeutic dose limits the problem of drug cross into the diseased cell. Moreover, the accumulation of the drug in healthy tissue can lead to serious side effects. Compared to healthy cells, rapidly proliferating cancer cells have a different energy metabolism, characterized by a high rate of glycolysis process, known as the Warburg effect [1]. To ensure a sufficient amount of nutrients up to this process, cancer cells have an increased demand for glucose. Therefore, the way to improve the selectivity of a biologically active compound concerning cancer cells can be its conjugation with a sugar molecule, which should facilitate the transport of such a compound through GLUT transporters [2,3].

Our studies focus on the use of small quinoline molecules as metal ion chelators and potential anti-cancer agents [4,5]. The presence of copper ions is important in many biological processes and is essential for cellular physiology in mammals. Changes in the homeostasis of copper ions play an important role in different types of diseases, such as inflammation, neurodegeneration as well as in carcinogenesis (copper is an essential cofactor for cancer growth and angiogenesis) [6]. Therefore, the use of metal chelators such as 8-hydroxyquinoline (8-HQ) seems to be an ideal way to control the level of copper in the organism [7]. We showed that 8-HQ derivative glycoconjugates containing 1,2,3-triazole fragment in the linker structure are able to form complexes with copper ions and potentially inhibit the multiplication of neoplastic cells by eliminating an important factor for their growth [5]. The synthesis of 8-HQ derivatives glycoconjugates is of particular interest due to their simple synthesis procedures, as well as facilitated intermembrane transport and improved solubility.

In these studies, we will use 1-thioglycosides for the synthesis of quinoline glycoconjugates (Figure 1). It is expected that the introduction of a sulfur atom instead of an oxygen atom into the anomeric position of the sugar will increase the stability of the obtained glycoconjugates against hydrolytic cleavage. Moreover, the introduction of an additional aminopyridyl fragment in the sugar aglycone should improve the cytotoxic activity of the final structure as their method of action may be related to better metal ion chelation. We will determine the antiproliferative activity of new derivatives based on the results of carried out cytotoxicity tests in the presence and absence of Cu^{2+} ions. The effect of the interaction of glycoconjugate complexes with metal ions on cell proliferation will be presented.

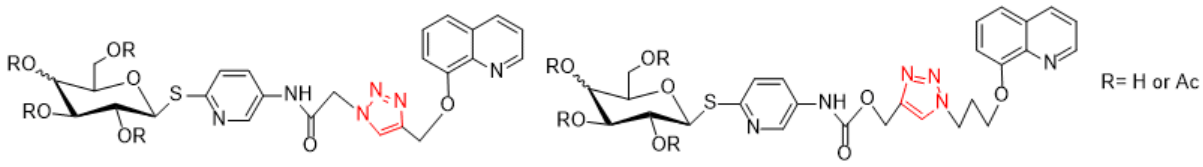

Figure 1. General structures of obtained glycoconjugates.

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NEW INSIGHTS INTO NKT CELLS ANTIGENS: NOVEL CERAMIDE DERIVATIVES SHOWED STRONG CELL STIMULATION

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Natural Killer T (NKT) cells stimulation has been largely studied over the past 2 decades, including several clinical trials, manly in cancer. Alpha-galactosylceramide (aGC) is the prototypical antigen for these cells and different families of derivatives or analogues were described seeking for NKT cell modulation. Although there are many evidences of NKT cell implication in immunological responses in a wide range of diseases, the cell over-stimulation potency of aGC together with the induction of Th1 and Th2 response put down the expectations of this compound. A more precise potency modulation and response type induction would lead to a promising candidate to use NKT cells as immune system stimulation in cancer therapies, as a vaccine adjuvant, autoimmune diseases, among others.

A family of antigens with novel chemical structure able to stimulate NKT cells is presented. This family have replaced the glycoside with a minimal structure. In spite of the apparent simplicity of the new structures, these analogues keep the ability to stimulate NKT cells. This new family showed a notable increase of potency when tested in human NKT cells, reaching a comparable NKT cells stimulation to that of aGC. Up to now, only glycosidic analogues or sugar mimetic compounds were reported active, pointing to a minimal polar footprint as key interactions. These compounds have potential for developing new ways to activate NKT cells, opening questions on the minimal polar interactions needed for glycolipid TCR-CD1d recognition.

TARGET SYNTHESIS OF DIARYLISOXAZOLES AS TUBULIN POLYMERIZATION INHIBITORS

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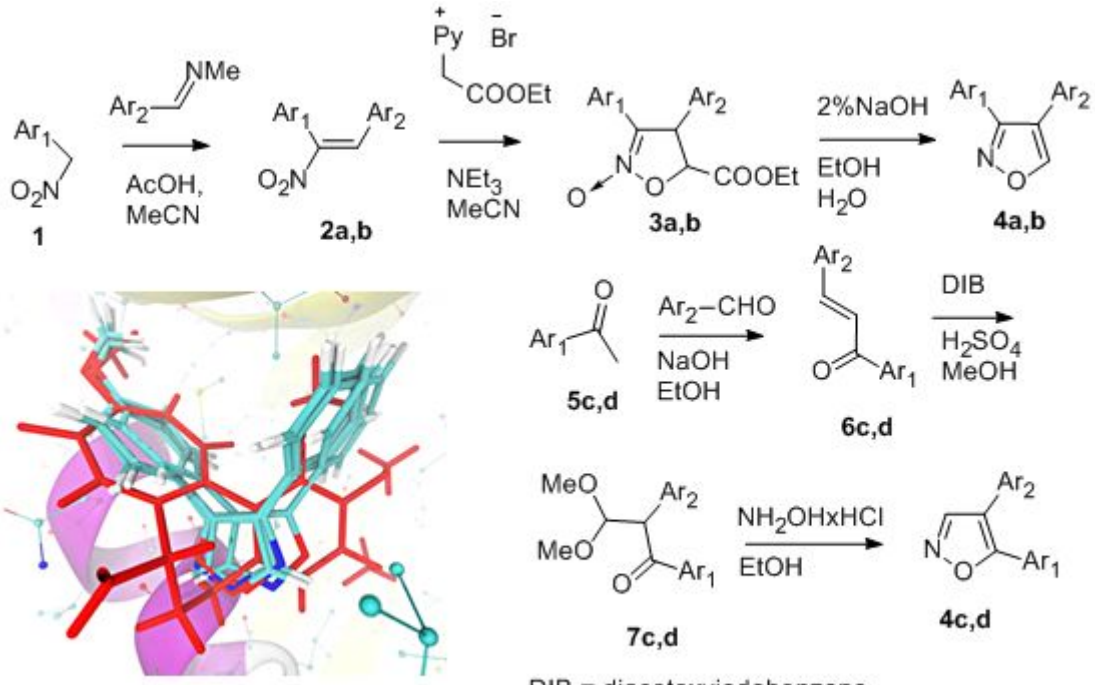
Tubulin is a reliable target in cancer therapy and besides some of the antitubulin compounds are used as anthelmintic and antifungal drugs. Among the variety of synthesized compounds[1], the presence of the trismethoxyphenyl group often provide good antitubulin activity. As part of the search for new anticancer drug candidates, computational modeling was carried out and high binding affinity was predicted for mono-methoxyaryl substituted isoxazoles. Using commercially available reagents and modified procedures we were obtained four isoxazole isomers.



Synthesized molecules were examined in sea urchin embryo assay for antitubulin activity and compounds **4a** and **4c** showed grater activity than isomers **4b** and **4d** as it was predicted in calculations using FEP and MD/FEP methods. Hence trismethoxyphenyl group is not necessary for nanomolar binding to a tubulin colchicine site.

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COVALENT FRAGMENTS DEVELOPMENT FOR CYSTEINE DRUG DISCOVERY

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Targeted covalent inhibitors and chemical probes have become integral parts of drug discovery approaches. In recent years, the number of drug candidates with a covalent mechanism of action progressing through clinical trials or being approved by the FDA has grown significantly; around 30 % of the marketed drugs are covalent inhibitors. This kind of inhibition has many desirable features, including higher biochemical efficiency of target disruption, less sensitivity toward pharmacokinetic parameters and increased duration of action that outlasts the pharmacokinetics of the compound [1].

The design of selective covalent, irreversible inhibitors is conceptually very attractive, but in practice hard to achieve. That is because it is challenging to strike the right balance of molecular properties between reactivity and selectivity [2-4]. In addition to the general practices of the FBDD paradigm, the design of electrophilic fragments requires consideration of the reactivity, reversibility, stability, synthetic accessibility, and size of the electrophilic functionality [2].

Taking into account a growing interest and widespread use of fragment-based drug discovery, we have designed an exclusive collection of covalent inhibitor fragments. A preliminary set of 4058 covalent fragments was created by singling out compounds with specific structural fragments (functional groups, warheads) that are known to form covalent bonds with amino acid residues in binding sites of targeted proteins, e.g., Lys, Cys, Ser, His and Tyr. Functional group analysis was carried out and their covalent property was confirmed based on alanine scanning. Next stage, molecules with highly reactive electrophilic and nucleophilic groups, as well as compounds with non-drug-like cores, were discarded as non-selective covalent binders. Such as combined approach has reduced previously set and analyzed only 1260 Cys-associated covalent compounds. But, finished set has not been made Ro5 compliant as it would have filtered out many small peptide-mimicking compounds.

Due to development approach, 157 non-patented compounds were selected according to the SciFinder database. Molecular docking algorithm was developed for cysteine covalent compounds of different specificity with selective proteins. Verified results will be presented on the poster.

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IDENTIFICATION OF NEW CANTHARIDIN DERIVATIVES IN SEARCH OF NOVEL POTENTIAL INHIBITORS OF PROTEIN PHOSPHATASES 2 AND 5 (PP2A, PP5)

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Cantharidin, a naturally occurring compound from the medicinal insect blister beetle (*Mylabris phalerata Pallas)* , and its water-soluble demethylated synthetic analog norcantharidin (better known as endothall), historically used in the purification of phosphorylated proteins, were identified as inhibitors of serine-threonine protein phosphatases (PPP). Cantharidin (CID 5944, hydrolyzing into cathartic acid - CID 2544) is a potent selective inhibitor of PP2A and PP5 [1], while norcantharidin (CID 93004) is a medium-strength inhibitor of PP2A only [2-3]. These compounds have been extensively studied as promising leads in the development of more effective therapeutics for the treatment of cancer, neurodegenerative disorders, and type 1 diabetes mellitus. As no highly PP5 selective inhibitors have been reported, the design and evaluation of novel biologically active derivatives of known inhibitors are of extreme importance.

Based on the template structures of human PP5 (RCSB PDB: 3H68, 3H63, 3H67, 3H62, 3H61, 3H69, 3H64) in complex with cantharidin and endothall and PP2A (UniprotKB: Q07099), we reconstructed its full 3-D model in Schrödinger ProtPrep. The ligands for docking input were prepared using CCDC Hermes. Cantharidin and endothall binding sites in PP5 and PP2A showed high similarity for protein phosphatases of different origin.

The docking results showed that 3 amino acids (His304, Asn303, Arg275) and (Arg400, His304, Arg275) were involved in the ligand binding on the surface of the protein phosphatases. In total, nine amino acid residues were demonstrated to be involved in the binding of the inhibitors. The completeness of the amino acid sequence of the experimentally obtained PP was determined by the method of pairwise alignment with the original sequence (UniprotKB: P53041) in the software package ClustalX 2.1. The binding site for PP5 was specified in 15 Å radius about -NE2 (HIS125).

We have selected over 50 derivatives of cantharidin from PubChem Database (pubchem.ncbi.nlm.nih.gov) and the Life Chemicals Chelator Focused Library with chemoinformatic analysis. Complexes with those cantharidin derivatives were predicted by flexible docking and evaluated based on CCDC GOLD scoring functions and results of molecular dynamics in GROMACS. As a result, we identified around 10 novel potential PP5 and PP2A inhibitors, previously not described in the literature.

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STUDY ON AMPK ACTIVATING EFFECT OF 3-BENZYLIDENE OXINDOLES

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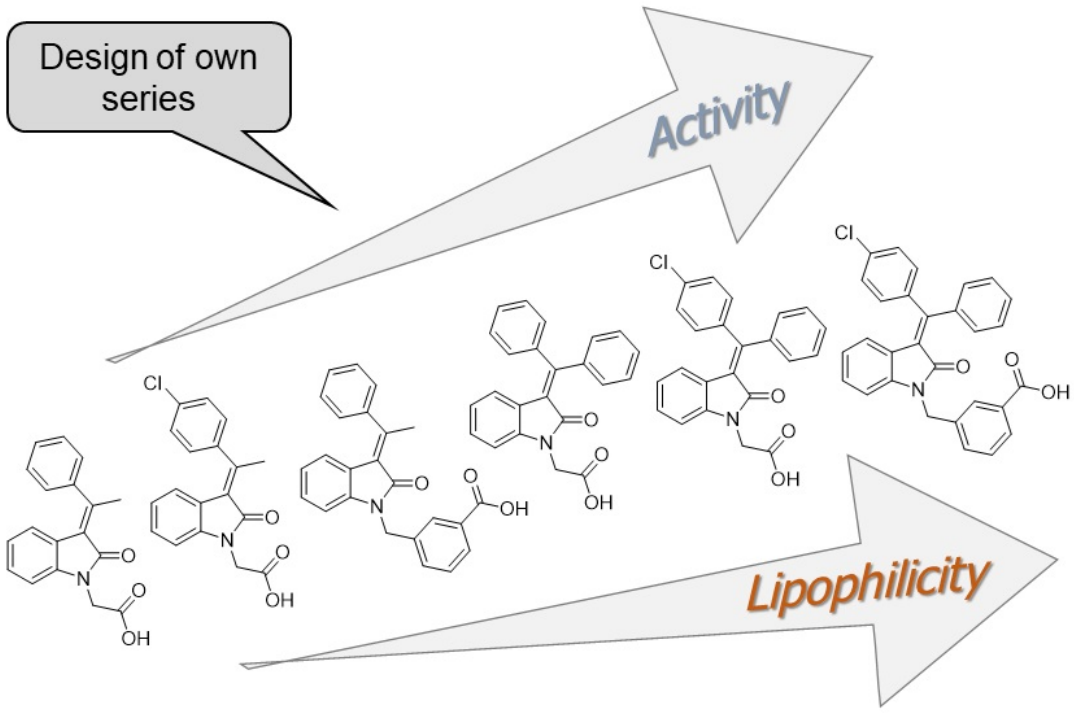
AMP-activated protein kinase (AMPK) is intensively studied last decades since it is down regulated in metabolic disorders such as diabetes and atherosclerosis, and its activation represents a therapeutic strategy for the treatment of such diseases. A number of compounds was found to activate AMPK through different mechanisms. Among the reported structures, a series of active compounds based on 3-benzylidene oxindole scaffold was shown to stimulate AMPK activation in vitro and in vivo [1, 2].

Here we studied the binding of 3-benzylidene oxindoles to the kinase domain of the AMPK α-subunit, which is thought to prevent its interaction with the autoinhibitory domain and thus result in AMPK activation. For this purpose, we developed cellular test system based on AMPKAR plasmid, which implements FRET effect, and synthesized several 3-benzylidene oxindole compounds with different substituents to obtain a series of AMPK activators with varying activity. Then their binding to various sites of the kinase domain was simulated. The most probable binding site for the studied compounds was established by the correlation of calculated and experimental data. The obtained results allow to analyze various classes of AMPK activators using virtual and high-content screening.



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EXPLORING THE CHEMICAL SPACE OF SIRT2 INHIBITORS THROUGH BIOMOLECULAR SIMULATIONS

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Modulation of activity of epigenetic eraser, NAD-dependent protein deacetylase sirtuin 2 (SIRT2), recently emerged as promising therapeutic strategy for the treatment of many diseases. Contrary to therapeutic potential, none of SIRT2 inhibitors reported to date have been approved for the market. Flexibility and hydrophobicity of SIRT2 inhibitor binding site were identified as main obstacles in structure-based (SB) drug design campaigns so far [1,2].

The Aims of this study were to explore conformational landscape of SIRT2-inhibitor complexes using molecular dynamics (MD) simulations, and to develop virtual screening (VS) workflow able to capture currently unexplored portions of SIRT2 inhibitors chemical space.

Total of 2μs of MD production run was performed representing the longest simulation performed on SIRT2 so far. Conformations obtained through essential dynamics analysis of MD trajectory were used in development of advanced SB VS workflow. Tenfold increase in early enrichment factors, compared to SB VS workflows using only X-ray structures, was the most prominent result of this study, suggesting these conformations could be true bioactive conformations of SIRT2-inhibitor complexes (Figure 1).

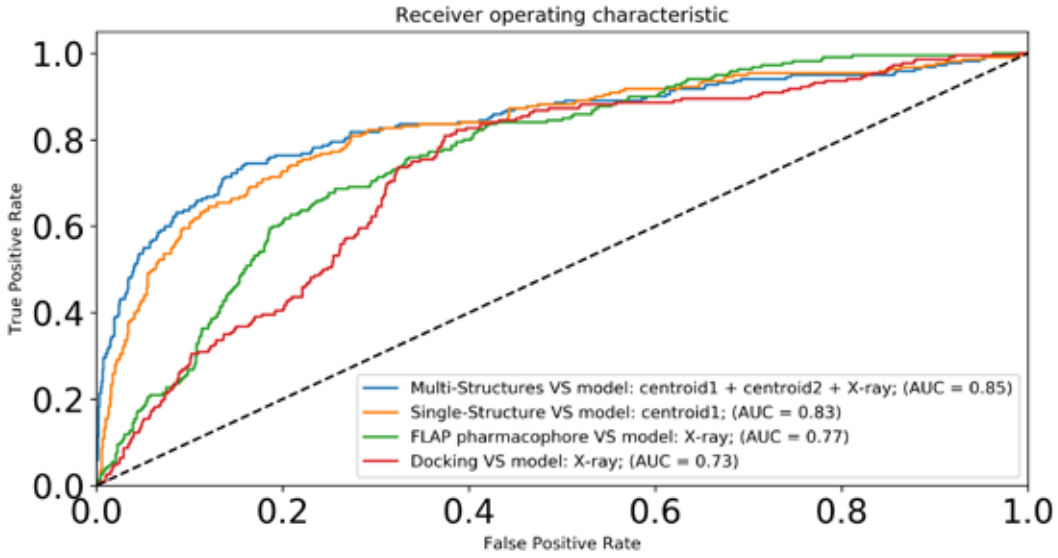

Figure 1. Results of the retrospective virtual screening.

Following the retrospective validation, commercially available databases were screened using novel VS model and top-ranked candidates were examined in in vitro enzymatic assays. For the two of the most potent and selective compounds, IC50 values were determined. Additionally, one of the tested compounds showed bromodomain-4 (BRD4) inhibition activity, opening the exciting avenue of the discovery of dual SIRT2/BRD4 inhibitors.

In conclusion, we developed and experimentally validated computational protocol for the discovery of novel SIRT2 inhibitors. Two novel chemotypes of SIRT2 inhibitors were identified and one compound, which could be first-in-class dual SIRT2/BRD4 inhibitor, was described.

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A SIMPLE OPEN SOURCE BIOINFORMATIC METHODOLOGY FOR INITIAL EXPLORATION OF GPCR LIGANDS' AGONISTIC/ANTAGONISTIC PROPERTIES

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Drug development is an arduous procedure, necessitating the testing of a large number of compounds. Therefore, any method providing an initial screening of molecules could limit the number of hits in drug discovery, prior to in vitro and in vivo validation, lowering the time and the money spend. We present a method which identifies potential molecules with agonistic and/or antagonistic properties on GPCR receptors by integrating receptor-ligand binding testing, with the knowledge on signalling events triggered by receptor activation (GPCRs-$G_{\alpha,\beta,\gamma}$ proteins binding and Gα activation, exchanging GDP for GTP). We show that, by integrating GPCR-ligand and Gα-GDP or -GTP binding, and simulating crystallographic data with high precision, we can correctly classify agonists, partial agonists, and antagonists, through a linear function, based on the ΔG (Gibbs free energy) of liganded-GPCR/Gα-GDP. We built our method using free online and locally executed software and testing known ligands on their receptors. We included two $G_{\alpha s}$ (β_2-adrenergic and prostaglandin-D_2), four $G_{\alpha i}$ (μ-opioid, dopamine-D_3, adenosine-A1, rhodopsin) and one $G_{\alpha o}$ (serotonin) receptors. Subsequently, we validated our methodology with a series of ligands on the recently deorphanized $G_{\alpha i}$ receptor OXER1. Our analysis shows that agonists induce a significantly higher affinity for the liganded receptor Gα-GDP interaction (as expressed by the ΔG for the binding complex). This affinity decreases when the same G-protein is bound to GTP, expressing the biologically relevant dissociation of the GTP-bound G-protein from the receptor and the subsequent intracellular signalling events. Our findings clearly show that, by integrating sequential steps of receptor downstream signalling in ligand-GPCR simulations (GDP-G_{α}binding), we can correctly predict the nature (agonist, antagonist, partial agonist) of a given small molecule. This approach, combined to properly implemented and successfully validated QSAR methods, may represent a useful addition to current research processes for the initial prediction and design of novel GPRC-interacting molecules.

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INHIBITORS OF MELATONIN RECEPTOR SUBTYPE MT3 IN THE TREATMENT OF GLAUCOMA

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Glaucoma is a neurodegenerative eye disease responsible for 15% of blindness worldwide. One of the crucial factors of this disease is the increased intraocular pressure (IOP). All available anti-glaucoma medications act only as IOP lowering agents. However, we have discovered that 2-oxindoles, being the ligands of the quinone reductase II (QR2, putative melatonin MT3 receptor), not only significantly reduce IOP, but also possess antioxidant neuroprotective properties(a-c). We have developed effective synthetic method for the preparation of oxindole-based melatonin bioisosteres(e-d). More than 75 new compounds were tested in vivo on normotensive rabbits. A group of compounds with high IOP reducing effect (>40%) at low concentrations (0.1 wt%) and prolonged action (up to 28 h) was identified(a). The obtained lead compounds are even less toxic than melatonin (LD50 = 2400 mg/kg and 800 mg/kg, respectively)(a). All tested compounds have great antioxidant properties – 100 times higher than melatonin. These results allow us to state that we are on the way to developing a new generation anti-glaucoma drug.

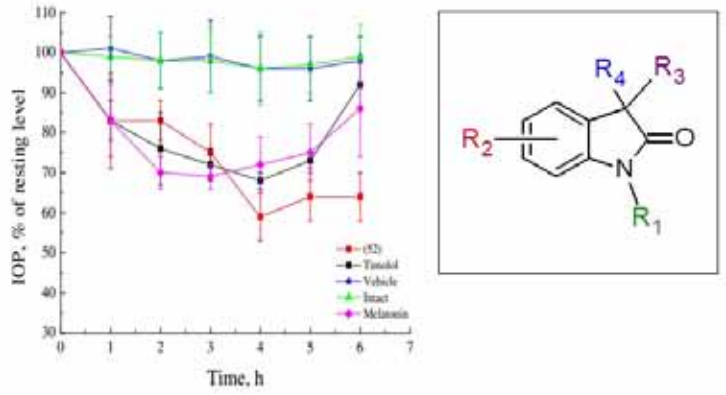

Fig. 1. Time-dependent study for (5-acetamido-2-oxindole-3-yl)acetic acid (52).

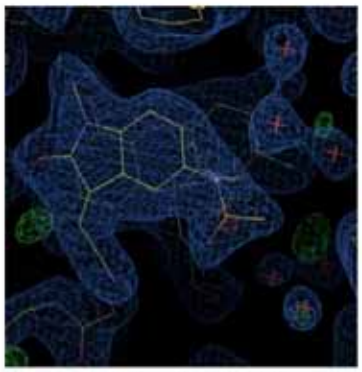

Fig. 2. X-ray crystal structures of (5-acetamido-2- oxoindolin-3-yl) acetonitrile in complex with QR2 (PDB ID: 4GQI, 4GR9).

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(Acknowledgments) This work was supported by the Russian Foundation for Basic Research (Project 20-03-00915A)

CARTILEGE REPAIR WITH THE USE OF A FUNCTIONALIZED SELF-ASSEMBLING PEPTIDE SCAFOLDS

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Regenerative medicine encompasses an interdisciplinary biomimetic approach to cell therapy and tissue regeneration using a triad of cells, scaffolds, and / or signals. A key element in this field of science is the development of scaffolds to reflect the three-dimensional nano-environment of the extracellular matrix. Peptide hydrogels are scaffolds which, from the point of view of tissue engineering, have great utility potential. Due to the unique biocompatibility, ease of modification and desirable physical properties, they are the material of choice for many applications in regenerative medicine [1]. Studies have shown that self-assembling peptide nanofibers have a beneficial effect on the regenerative processes of the tissues of the human body [2]. Peptide hydrogels are systems composed of a three-dimensional network of peptide chains and water filling the spaces between them. They combine spontaneously to form nanofibers with a fiber diameter of the order of 10nm. Hydrogels contain over 99% of water. Their macroscopic structure is similar to the naturally occurring extracellular matrices of collagen [3]. These substances form three-dimensional structures the so-called. scaffolds, which support the differentiation and proliferation of stem cells and, as a result, regeneration of damaged tissue [4].

One of the best-characterized peptide hydrogels is the RADA16-I peptide (Ac-RADARADARADARADA-NH2). It is made up of four tetrapeptide repeats containing arginine (R), alanine (A) and aspartic acid (D), which organize in the water environment into the structure of β-sheets. The secondary structures of the RADA16-I peptide are characterized by the presence of hydrophobic and hydrophilic surfaces and create organized structures of supramolecular fibers, stable in a wide range of temperatures and denaturing agents. Due to the possibility of connecting functional fragments to the C-terminus, they have been widely used in regenerative medicine of such tissues as: nervous, bone and cartilage [5].

The aim of our research is to develop innovative hydrogel peptide, which as a result of functionalisation will find application in the medicine of regenerative cartilage as scafold of substances with confirmed biological activity. For this purpose, a peptide consisting of the rada 16 peptide hydrogel, a sequence specific for the enzyme metalloproteinase 7 and an active sequence being a fragment of the bone morphogenetic protein BMP 2 was designed.

ACKNOWLEDGEMENT

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ALLOSTERIC MODULATION OF D2LONG RECEPTOR IN COMPLEX WITH Gi1 AND Gi2 PROTEINS

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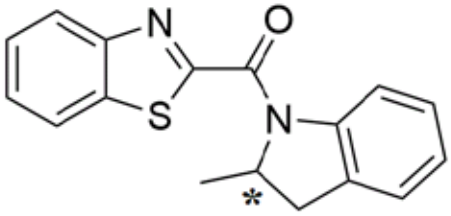
Dopamine D_2 receptor belongs to aminergic G protein-coupled receptors and it is involved in many diseases, including schizophrenia and Parkinson's disease. Thus, it plays an important role in the pathophysiology and treatment of psychosis and movement disorders.

One of the hot topics in GPCR-oriented drug discovery is development of allosteric modulators. The allosteric ligands are more promising as drugs comparing to compounds with classical orthosteric mode of action. Positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs) of dopamine D_2 receptor have been proposed for treatment of Parkinson's disease or schizophrenia. *In vitro* studies on benzothiazole racemic compound (figure below shows chemical structure of the allosteric modulators showing the racemic center) reported by Wood et al.[1] and its enantiomers showed that R enantiomer acts as a PAM by enhancing the effect of native ligand on G protein activation, cAMP signaling, and enhancing the binding of [^{3}H]-dopamine. The S enantiomer attenuates the effects of the R isomer and inhibits [^{3}H]-dopamine binding[1]. Here we present molecular docking and molecular dynamics simulations (MD) performed to investigate the allosteric effect of the R and S enantiomers of this compound on the D_{2LONG} receptor (the isoform with long intracellular IL3 loop) in complex with respective G proteins (G_{i1} or G_{i2}). We also designed a derivative of this compound, however in vitro studies it displayed no effect on dopamine D_2 receptor functioning and hypothetically it can act as a SAM (silent allosteric modulator) of this receptor. Due to a considerable toxicity of the studied derivative as found using MTT assay, it was not possible to investigate the effect of this compound *in vitro* at higher doses.



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DESIGN OF ANTIVIRAL COMPOUNDS AGAINST BUNYAVIRALES SUPPORTED BY LIGAND-NUCLEASE INTERACTIONS STUDIES

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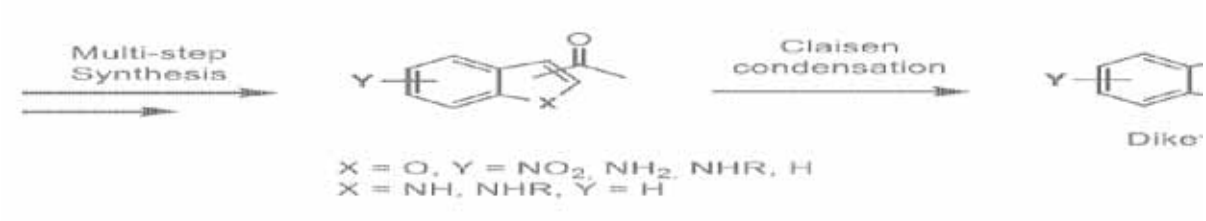
The World Health Organization classifies some of *Bunyavirales* genus as prioritized for R&D in public health emergency context due to their epidemic potential for humans and declares that whether there are non-available or insufficient countermeasures[1].

Bunyavirales are enveloped viruses with negative stranded RNA genome which encodes especially an endonuclease (EndoN). The latter is located in the N-terminal domain of the multifunctional viral (L) protein and it is highly conserved among different families of *Bunyavirales*. EndoN is crucial at the earlier stages of the viral transcription mechanism[2],[3]. Structural and functional studies demonstrated that the activity of the EndoN is Mn^{2+} and/or Mg^{2+} dependent.

Inhibition of the EndoN activity *via* metal chelation mechanism was recently validated as a good strategy by the approval of Baloxavir to treat the influenza infections[4]. In our laboratory, diketo acids (DKA) were described as metal chelators and potent inhibitors of *Bunyavirales* EndoN[5],[6],[7].

In order to extend our library, we explored the potential of Benzofuran- and Indole- DKAs as EndoN inhibitors. These heteroaromatic rings are frequent scaffolds in medicinal chemistry[8].

In this Ph.D. project, I explored the antiviral potential of 5-aminobenzofuranyl DKA (DABBA) and 2-Indolyl DKA (DIBA) derivatives. Synthetic routes were set up to provide methyl ketones, which are key synthons to generate diketo esters (DKE) and subsequent DKAs (scheme.1). Their efficiency was evaluated by biophysical, biochemical and minigenome assays.



Scheme.1 General synthetic route for DKA derivatives.

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THE SEARCH FOR CHOLINESTERASE INHIBITORS AMONG NOVEL HISTAMINE H3 RECEPTOR LIGANDS - 4'-[1,1'-BIPHENYL]-4-CARBONITRILE DERIVATIVES OF (HOMO)PIPERIDINE

Dorota Łażewska (1), Paula Zaręba (2), David Reiner (3), Marek Bajda (2), Annika Frank (3), Holger Stark (3), Barbara Malawska (2), Katarzyna Kieć-Kononowicz (1)

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The loss of cognitive function in Alzheimer's disease is connected with the damage of cholinergic transduction in the brain and lowering of acetylcholine levels. Enhanced cholinergic neurotransmission in the brain can be achieved by blocking of histamine H_3 receptor (H_3R) or through the combination of H_3R antagonism with e.g. acetylcholinesterase (AChE) and/or butyrylcholinesterase (BuChE) inhibition in a single molecule.

Recently, we have described a series of biphenyloxyalkyl derivatives as promising H_3R ligands (19 nM ≤ K_i < 270 nM). Among them compound **E-153**, (1-(5-(4-phenylphenoxy)pentyl)homopiperidine), showed high H_3R antagonist affinity (K_i = 33 nM) and butyrylcholinesterase (BuChE) inhibitory activity (IC_{50} = 588 nM). As a continuation of this work we have designed, synthesized and biologically evaluated a series of 4'-[1,1'-biphenyl]-4-carbonitrile derivatives of (un)substituted (homo)piperidine with different alkyl linker lengths (Fig.1).

In vitro evaluation of the series showed affinities for human H_3R stably expressed in HEK293 cells in nanomolar range (K_i < 300 nM). AChE and BuChE inhibitory activities were evaluated by spectrophotometrical Ellman's method using AChE from electric eel and BuChE from horse serum (2.5 units/1 mL). Most compounds showed inhibitory activity in low micromolar range for both cholinesterases (IC_{50} < 8 μM).

The kinetic studies of AChE and BuChE inhibition were performed for compound **E307** (1-(6-(4-(4'-carbonitrile)phenylphenoxy)hexyl)homopiperidine) one of the most potent cholinesterase inhibitor (AChE: IC_{50} = 1.5 μM; BuChE IC_{50} = 1.4 μM) in this series.

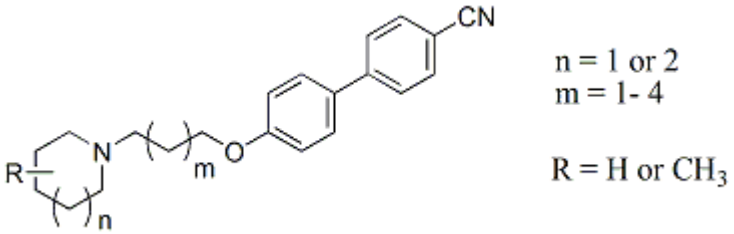

Figure 1: General structure of 4'-[1,1'-biphenyl]-4-carbonitrile derivatives.

Financial support by the National Science Center grant based on decisions No DEC-2016/23/B/NZ7/02327 is gratefully acknowledged as well as support by ERNEST COST Action CA18133.

REPLACEMENT OF THE AZEPANE RING IN CM304 IMPROVED THE IN VITRO METABOLIC STABILITY

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CM304, (3-(2-(azepan-1-yl)ethyl)-6-(3-fluoropropyl)benzo[d]thiazol-2(3H)-one), is a sigma-1 receptor (S1R) antagonist. It demonstrated promising antinociception in a battery of animal pain models with no significant liabilities compared to morphine and gabapentin.[1] Unfortunately, CM304 suffers from poor metabolic stability in rats ($t_{1/2\ in\ vitro}$ = 1.6 min, $t_{1/2\ in\ vivo}$ = 2.3 h) and poor bioavailability (F= 0.7%).[2] Metabolic soft spot identification of CM-304 revealed two soft spots: the azepane ring and the 6-(3-fluoropropyl) benzothiazolone moiety. Due to synthetic feasibility, and similarity in structure, S1R affinity, and metabolic stability, we decided to use the defluorinated analog SN56, (3-(2-(azepan-1-yl)ethyl)-6-propylbenzo[d]thiazol-2(3H)-one). SN56 showed high S1R affinity in rat liver membranes of 1.6 nM, moderate selectivity over sigma-2 receptor (169-fold),[3] and *in vitro* half-life of 1.0 min. Here, our goal is to optimize the azepane ring and induce metabolic shifting to reduce metabolic degradation, with a half-life cutoff >30 min, while keeping the affinity and selectivity at S1R.

We have investigated five strategies to increase the half-life. Among them are (1) blocking the metabolic labile site; (2) reducing the ring size; (3) altering the electronic properties; (4) altering the steric bulk around the protonatable nitrogen atom; (5) using two strategies of altering both the steric and electronic properties. Nineteen final compounds were synthesized and tested for their *in vitro* metabolic stability in rat liver microsomes. Interestingly, two compounds demonstrated significant improvements in their *in vitro* half-lives while maintaining moderate affinity at sigma-1 receptors. Moreover, initial *in vivo* screening of one of these compounds showed promising antinociception comparable to that demonstrated by CM304. The ED_{50} and the confidence interval is 5.81 (4.62-7.32) mg/kg, i.p. in the mouse formalin assay. Further evaluations are undergoing to test the efficacy of these optimized compounds in different pain assays as well as screening for possible liabilities.

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CHEMINFORMATICS ANALYSIS ON MOLECULAR DATASETS OF TRANSCRIPTION FACTORS ASSOCIATED WITH QUORUM SENSING IN PSEUDOMONAS AERUGINOSA

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New agents against *P. aeruginosa* is an urgent necessity, mainly due to resistance issues. In order to find the key features for the antagonism activity, we built and analyzed molecular datasets (DSs) of the transcription factors (TFs) LasR, PqsR and RhlR related to the quorum sensing system in *P. aeruginosa*. In silico approaches were done to find structural characteristics that allow us to explain the activity of a molecule. DSs were obtained from research articles, review papers, PubChem Bioassay [1] and ChEMBL [2]. For all DSs, we obtained representative scaffolds according to Bemis and Murcko definition [3]. A maximum common substructure analysis was done on the most frequent scaffolds for each DS. Activity cliffs were established using the Tanimoto metric by comparing different 2D fingerprints. Constellation plots and t-distributed stochastic neighbor embedding (t-SNE) were carried out for the representation of chemical space occupied by DSs. 289 molecules were analyzed, 188 belongs to LasR DS, 54 to PqsR, and 47 to RhlR. For all TFs some scaffolds were usually associated with molecules with antagonist activity. A maximum common substructure analysis shows that all DSs have molecules that, with small structural changes, display different types of biological activity (agonist, antagonist, and inactive). The activity cliffs analysis confirms that the molecule size is a key characteristic for LasR and RhlR antagonist activity, while hydrogen bond acceptors and donors are key for PqsR antagonist activity. All DSs are close in chemical space, especially those for LasR and RhlR, improving the possibilities to find a molecule with dual activity against them. Constellation plots give us the possibility to associate standard cores with biological activity and the number of compounds they have, finding that diphenyl rings have better activity against LasR. The size of molecules and the number of hydrogen bonds (acceptors and donors) have a key role for the activity against TFs. However, the structural similarity between agonists and antagonists makes it difficult to search for compounds with a specific activity.

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THE MECHANISM OF ALPHA MANGOSTIN COMPOUNDS TO INHIBIT THE MCF7 GROWTH BY INHIBIT THE OVER EXPRESSION RECEPTOR ESTROGEN AND AKT: WESTERN BLOT ASSAY AND MOLECULAR DYNAMIC SIMULATION

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α-mangostin is a yellow xanthone compound that isolated from Mangosteen pericarp (*Garcinia mangostana* L) which has been shown to have anti-inflammatory, antifungal, antimicrobial and anti-cancer activities. The purpose of this study was to determine the activity of α-mangostin compounds as anti-breast cancer against ERα, to know the cytotoxic activity of alpha mangostin, and to determine the mechanism of α-mangostin in inhibiting the growth of over-expression of ERα. The results of this study with molecular docking methods (molecular docking), showed that the free energy in α-mangostin against ERα was -8.55 kcal/mol while in tamoxifen -11.37 kcal/mol. Molecular dynamic results showed that DG of α-mangostin using MMGBSA was -42.704kcal/mol. The examination results of α-mangostin cytotoxic activity with resazurin method obtained IC_{50} values of 0.044 μg/mL, while the tamoxifen control 0,1 μg/mL. The test with western blot method, it was found that the Akt protein in the 2xIC_{50} band had decreased, whereas in ERα MCF7 breast cancer cells did not experience inhibition of proliferation. It can be concluded that α-mangostin could be a potential ERα inhibitor and breast anticancer based on molecular docking and cytotoxic testing. Based on western blot testing, α-mangostin can inhibit the proliferation of breast cancer from Akt but not to ERα.

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TRITERPENIC ACID AMIDES AS A PROMISING AGENT FOR TREATMENT OF METABOLIC SYNDROME

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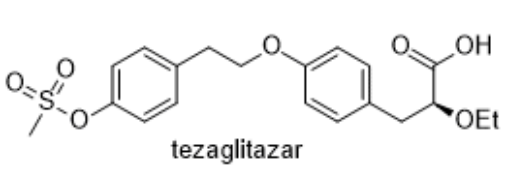
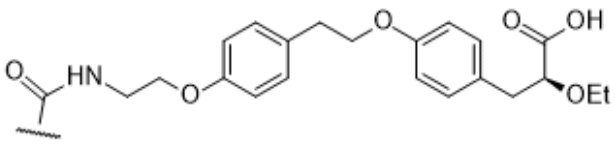
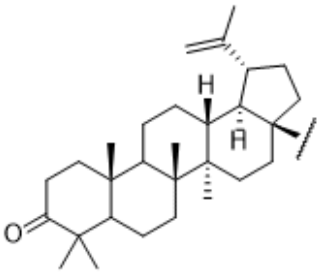
Type 2 diabetes mellitus is a disease of complex pathogenesis and pleiotropic clinical manifestations. It consists of an array of dysfunctions characterized by hyperglycemia and resulting from the combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion.

Over the past two decades, a subfamily of nuclear receptors activated by peroxisome proliferators (PPAR), has been considered as valuable pharmacological targets, the activation of which can normalize metabolic dysfunctions and reduce cardiovascular risk factors associated with type 2 diabetes. Double agonists PPAR-α,γ, combining in one molecule the metabolic and hypoglycemic properties of α and γ-agonists, have been proposed as a promising therapeutic strategy for the treatment of metabolic syndrome.

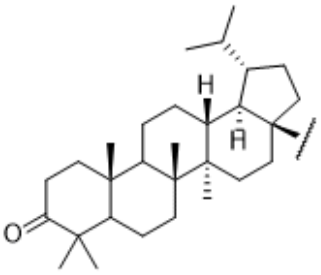


tezaglitazar

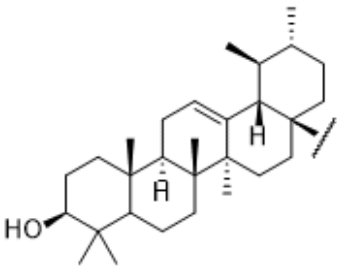




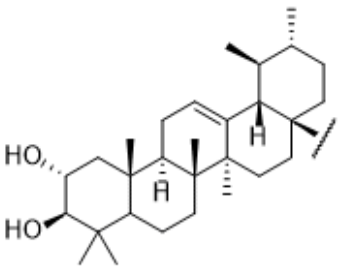
Betulonic



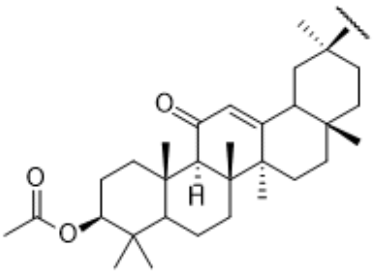
Dihydrobetulonic



Ursolic



Corosolic



18-β-Glycyrrhetinic

Based on tesaglitazar structure, we have synthesized a series of triterpenic acid amides with 2-etoxy-3-phenylpropanoic acid pharmacophoric fragment. Ursolic and betulonic derivatives were shown to improve glycemic control and lipid abnormalities in high fat diet mice C57/BL/6 in in vivo experiments.

This work was supported by the Russian Foundation for Basic Research project No. 19-03-00685.

EFFECT OF POINT MODIFICATIONS ON THE PROCESS OF OLIGOMERIZATION AND AGGREGATION OF HUMAN SERUM AMYLOID A

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Human Serum amyloid A (hSAA) has various functions in our body, which include, among others inflammation response and participation in cholesterol transport and metabolism. The concentration of this protein varies from 7-8 mg /l of plasma under physiological conditions to even 1000 mg /l in response to injuries, inflammation or infection.[1]

In pathological states, SAA obtain new properties and is able to form fibrillary aggregates. These aggregates are deposited as extracellular deposits in various organs are a burden that obstructs proper functioning of the affected tissues.[2] It is therefore very important to find an answer to the question of what is the mechanism of SAA aggregation and how it can be prevented.

The polypeptide chain of the human SAA consists of 104 amino acid residues.[3] As the native form of the protein is quite stable and it is difficult to observe aggregation process on it, we decided to use less stable isoforms for our research, in which the oligomerization / aggregation process is much faster, so easier to observe.

The isoforms to used our research have point modifications, such as introduced methionine residue at position -1 or replacement of residues at position 60 (D → N) and 71 (H → R). These are slight modifications in the SAA sequence, but they significantly affect the oligomerization properties of individual isoforms.

Acknowledgments
This scientific work was financed under the program "Diamond Grant", as a research project DI2015 022445.

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USNIC ACID ENAMINES WITH TERPENE SUBSTITUENTS AS POTENTIAL Tdp1 INHIBITORS

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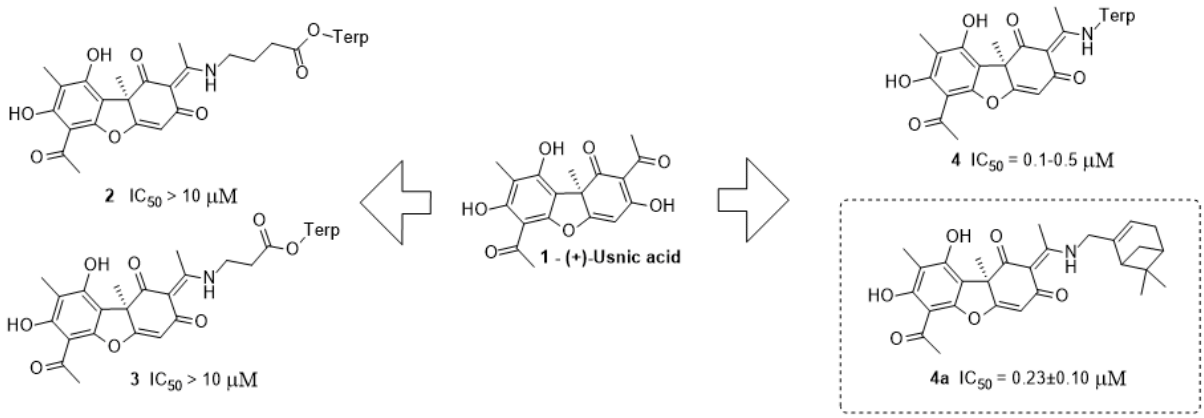
Tyrosyl-DNA phosphodiesterase 1 (Tdp1) is one of the enzymes that play a key role in removing DNA damage resulting from inhibition of Top1 by anticancer drugs camptothecin and its bioavailable derivatives topotecan and irinotecan. There is the hypothesis that Tdp1 is responsible for drug resistance in some cancers and that the combination of anticancer drugs and Tdp1 inhibitors can significantly increase the effectiveness of chemotherapy [1].

In [2] we showed that enamine derivatives of (+)-usnic acid (UA) are effective inhibitors of Tdp1, have low toxicity for human breast adenocarcinoma cell line MCF-7 and enhance the cytotoxic effect of camptothecin *in vitro*. Also, it is known that for another type compounds based on (+)-UA replacing of the aromatic substituent with terpene one resulted in a doubling of the inhibitory activity [3]. We synthesized new enamine derivatives of (+)-UA with different terpene fragments (linear, mono- and bicyclic) connected with natural frame via an aminoacid linkers (**2** and **3**) or directly (**4**).



Real-time measurement of Tdp1 cleavage activity test based on fluorophore/quencher-coupled DNA-biosensor showed, that only compounds **4** are active against Tdp1 in the submicromolar range of concentrations. The most promising compound **4a** which was synthesized from (+)-UA and bicyclic myrthenyl amine has IC_{50} 0.23 mM.

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THE EFFECT OF 3,7-DIAZABICYCLO[3.3.1]NONANES WITH MONOTERPENOID MOIETIES ON PHYSICAL PERFORMANCE OF TESTING ANIMALS

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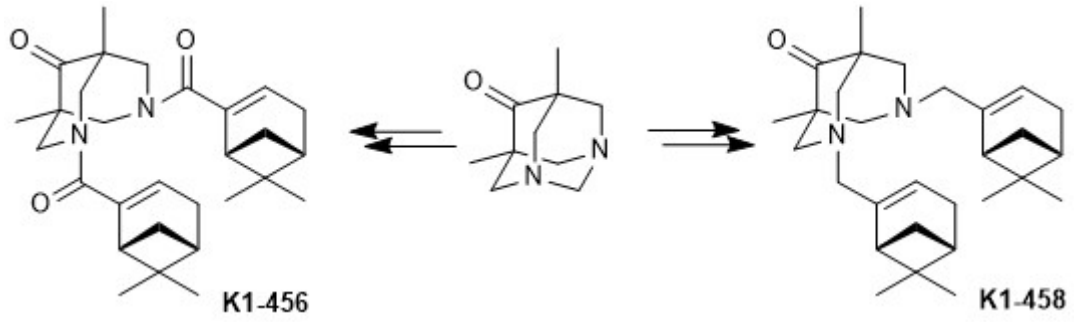
Currently, increasing physical performance is an urgent task, not only in the field of extreme conditions, for the purposes of sports and military medicine, but also in the pharmacology of a healthy person. We have synthesized a number of monoterpene derivatives with 3,7-diazabicyclo[3.3.1]nonane (bispidine) scaffold for subsequent study of their effect on the physical endurance of mice.



Physical endurance has been studied using two tests: exhaustive swimming with a load of 7% of body weight and exhaustive treadmill running. It has been shown for the first time that derivatives of 3,7-diazabicyclo[3.3.1]nonane moiety which contain (–)-myrtenal residues have a stimulatory effect on the performance of mice, which exceeds the effect of the reference drug bromantane. Compound K1-458 containing monoterpenoid residues linked to a bispidine fragment via amino groups is the most effective in increasing the duration of running (35%) and swimming (20%) time at a dose of 100 mg/kg after single intragastric administration. It has been also established that the LD_{50} for these compounds exceeds 1000 mg/kg [1].

Authors are grateful to the Russian Foundation for Basic Research (Grant No. 18-03-00437) for the financial support.

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DESIGN, SYNTHESIS AND EVALUATION OF A PEPTIDE CONJUGATE TO PROTECT BIOMATERIALS FROM UNDESIRED IMMUNE ATTACK

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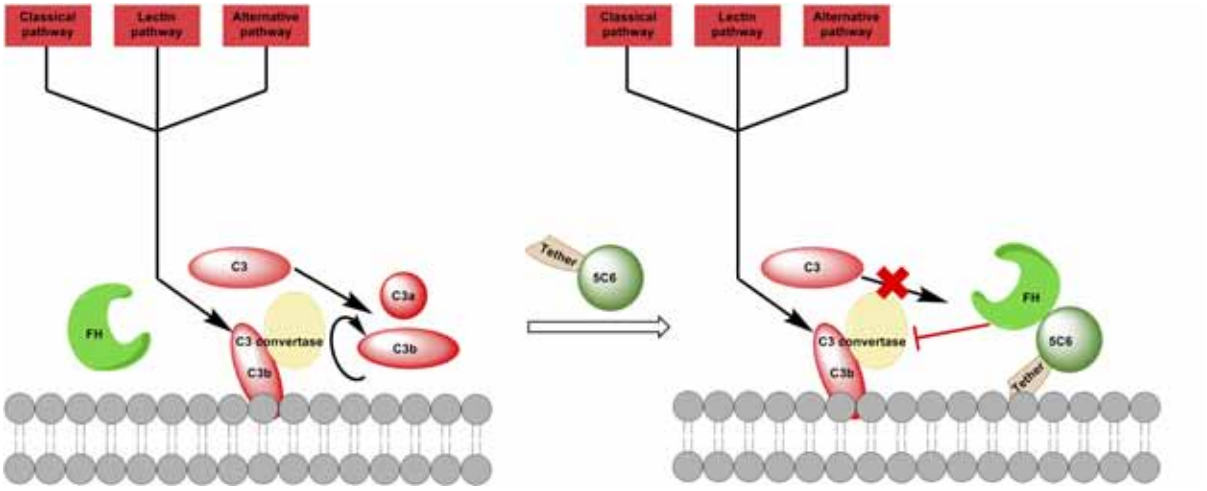
Although important progress has been made to protect biomaterials such as transplants, implants or liposomes from undesired host immune recognition and attack, several problems remain unsolved. One of these is the involvement of the complement system, the humoral part of innate immunity. It broadly and swiftly recognises non-self surfaces, leading to direct cell damage and induction of the adaptive and cellular innate immune system. In order to restrict complement activation to non-self or degenerated surfaces, the organism tightly controls complement through regulators in solution and on surfaces. One promising approach to protect biomaterials from immune attack, inspired by microbial immune evasion, is to specifically recruit these large molecular complement regulators with small to medium-sized molecules to surfaces and to prevent complement attack *in situ*.

Pursuing this idea, a disulphide-bridged cyclic peptide (5C6) was previously discovered by our group through phage display screening. 5C6 showed nanomolar binding affinity to the plasma-borne, major complement regulator Factor H (FH). Attractively, FH inhibits complement's central self-amplificatory C3 convertases where all three activation pathways converge. 5C6 could reduce complement activation when combined with appropriate tethering motifs by acting as a bridge between FH and model surfaces (Fig. 1). [1, 2]



Based on this initial hit, comprehensive structure-activity relationship studies were conducted in which all residues having been identified for being important for the FH-5C6 interaction were investigated through replacement with commercially available or tailor-made building blocks. Furthermore, changes in the macrocycle size and efforts to replace the disulphide by a more bioinert functional group allowed us to further improve the properties of 5C6. Moreover, the ideal position for the tether conjugation was determined by ELISA and biophysical methods. Finally, functional tests in clinically relevant models are currently being undertaken to assess the translational significance of these findings.

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3-SUBSTITUTED 2-OXINDOLE DERIVATIVES AS POTENTIAL ANTI-DIABETIC AND ANTITUMOR AGENTS

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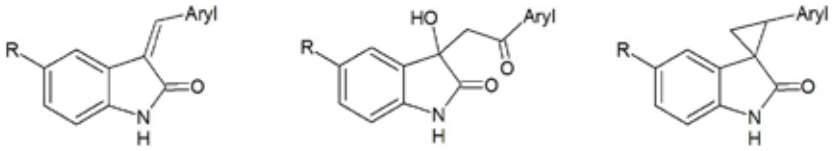
Inhibition of glycogen synthase kinase 3β *(*GSK-3β*)* is a new and widely investigated approach to the treatment of diabetes mellitus [a], cancer and Alzheimer's disease. We report the synthesis of novel GSK-3β inhibitors with pronounced antioxidant, anti-inflammatory and antitumor activity [b]. Over 50 3-substituted 2-oxindole derivatives were synthesized and tested *in vitro* against GSK-3β and α-glucosidase, another molecular targets linked with diabetes [c].



The convenient choice of 2-oxindole scaffold allowed for the wide range of substituent variation which resulted in the possibility of effective and selective binding to both molecular targets. Lead compounds were shown to inhibit GSK-3β and α-glucosidase in a cell-based assay with IC_{50} 4.19 nM and 6.78 μM respectively with low cytotoxicity.

Oral glucose tolerance test in rat model of type 2 diabetes mellitus demonstrated prominent antihyperglycemic activity of 3-substituted 2-oxindole derivatives.

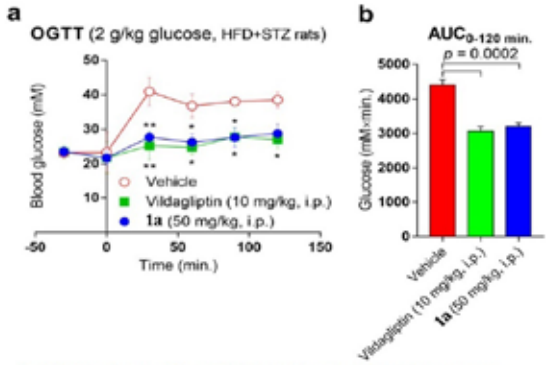


Fig. 1. Effect of compound **1a** and DPP-4 inhibitor vildagliptin on glucose response during an oral glucose tolerance test in HFD+STZ diabetic rats.

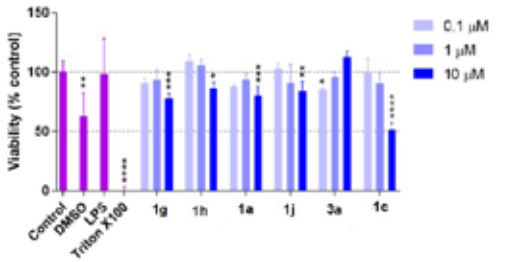


Fig. 2. Assessment of cytotoxicity in human PBMCs

GSK-3β inhibitors are proven to successfully combat systemic inflammatory process and oxidative stress, factors crucial in the development of type-2 diabetes **[d]** Obtained substances were able to significantly reduce iNOS activity in LPS-induced macrophages, showcasing their anti-inflammatory properties. Novel 3-arylidene 2-oxindole derivatives were tested *in vitro* on lung adenocarcinoma (A549) and colon cancer (HCT116) cell lines and displayed cytotoxicity in the low micromolar range. Key substitutes were found both for compounds exhibiting cytotoxicity in the low micromolar range and compounds with antidiabetic properties.

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(Acknowledgments) This work was supported by the Russian Foundation for Basic Research (Project 20-03-00915A)

DESIGN, SYNTHESIS AND BIOLOGICAL CHARACTERIZATION OF PHOTO-REGULATED MELATONIN RECEPTOR LIGANDS IN TYPE 2 DIABETES AND NEURODEGENERATIVE DISEASES

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Melatonin regulates a big variety of physiological and neuroendocrine functions, and it has well-established neuroprotective properties. Consequently, its deregulation is associated with many metabolic, autoimmune and neurodegenerative diseases, although its specific molecular mechanism still remains elusive. Emerging evidence shows that GPCR signaling can be prolonged or initiated not only in the plasma membrane but also in intracellular compartments.[1] Therefore, the interplay between cell surface and intracellular melatonin receptors needs to be characterized.

Innovative pharmacological tools based on photo-regulated melatonin ligands have been synthesized. These structures have been designed to be inactive at the level of receptor, but able to be activated by using specific light conditions. Among these molecules, caged compounds and azobenzenes are found. For caged-type derivatives, light triggers a photolytic reaction that separates the caging moiety from active melatonin, which is released at a specific intracellular location. For azobenzene-type derivatives, light switches reversibly the structure of the ligand derivative, in such a way that the new conformation is ideally recognized by the receptor. Therefore, these tools allow a precise temporal and spatial control of melatonin receptors activation upon illumination.

Functional and affinity assays have been performed on the first family of melatonin-caged compounds. According to the obtained results, all tested ligands show a notable loss of activity before light irradiation, as well as lower affinity to the receptors in comparison to melatonin (at least 2-log shift decrease). Likewise, they show the expected behavior on the receptors upon light activation, in terms of affinity and activity for MT1 and MT2. This observation is notable for compound **MCS-0382**, which has been selected for the development of a molecule that could reach intracellular melatonin receptors. In conclusion, the first family of photo-regulated melatonin ligands for controlled temporal and spatial receptor activation has been designed. These innovative chemical tools will help to understand the role of mitochondrial melatonin receptors and clarify their role in metabolic and neurodegenerative diseases.

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PUTTING EFFORTS FOR RECOGNIZING SOME NATURAL PRODUCTS AS SARS-COV-2 SPIKE GLYCOPROTEIN INHIBITORS: PROBABLE SOLUTIONS TO THIS COVID-19 SCENARIO

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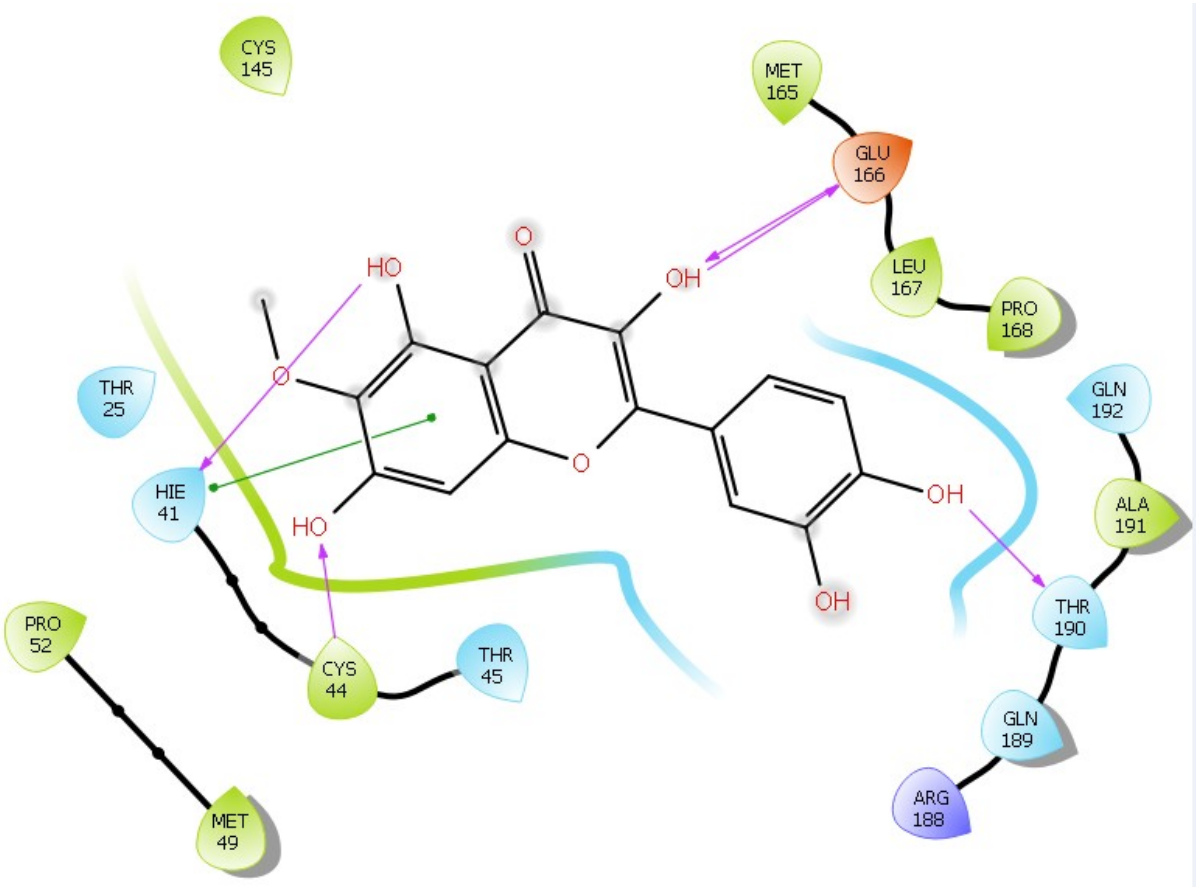
After the discovery of one of the imperative therapeutic targets of coronavirus, “severe acute respiratory syndrome coronavirus-2 spike glycoprotein (viral SARS-CoV-2 spike glycoprotein) S1 subunit”; that mediates the viral infection by promoting the entry of the virus into the human cells through binding phenomenon with the cellular membrane. We put our best efforts in discovering or rather recognizing several potential natural products-based low molecular weight ligands (LMWL) inhibitors from diverse classes like flavonoids, chalcones, aurones, phytosterols, etc. against the above target through induced-fit molecular docking techniques, using Maestro 9.1 (Schrodinger) software [1-2] (Glide module) along with the pharmacokinetic predictions (33 parameters). The docking scores of natural compounds were reported and a probable conclusion for pharmacotherapeutics was drawn from this exploration. This study will positively motivate the global enthusiastic researchers to rationally develop positive solutions against the COVID-19 attack through the discovery of therapeutically privileged compounds where even vaccines failed to show impressive results.



KEYWORDS: Coronavirus, COVID-19, SARS-CoV-2 Spike Glycoprotein, Natural, Inhibitor, Molecular Docking

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SYNTHESIS AND NITRIC OXIDE (NO) PRODUCTION INHIBITION ACTIVITIES OF 1,3,6-TRIHYDROXYXANTHONE AND ITS NEW AMINE DERIVATIVES

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Inflammation is a response to the immune system to protect our body from the infection of foreign organisms, such as bacteria and viruses. However, uncontrolled acute inflammation may lead to variety of chronic inflammations which are harmful, causing life threatening hypersensitivity reactions and progressive organ damage. Xanthone is one of the secondary metabolites isolated from plant that found to exhibit anti-inflammatory activities and potential to be the lead compound for anti-inflammatory drugs. The starting compound, 1,3,6-trihydroxyxanthone was synthesized *via* the condensation of 2,4-dihydroxybenzoic acid and phloroglucinol in the presence of Eaton's reagent. Five tertiary amine-substituted xanthone derivatives were synthesized by alkylation with 1,4-dibromobutane in the presence of K_2CO_3 as the catalyst, followed by amination with secondary amine under reflux condition. The synthesized xanthones were purified by chromatography techniques and structurally characterized by spectroscopy methods including nuclear magnetic resonance (NMR), mass spectrometry (MS) and Fourier-transform infrared spectroscopy (FTIR). The anti-inflammatory activities of synthesized xanthones were evaluated by inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells. The standard drug used was diclofenac sodium which showed 79% NO production inhibition at the concentration of 300 μM. Among the synthesized amine-substituted xanthone derivatives, xanthone with 2-fluoro-N-methylbenzylamine substituent showed the highest NO production inhibition (70%) at the concentration of 100 μM. The results suggest that amine-substituted xanthone derivatives may be useful in preventing inflammatory diseases mediated by excessive production of NO. Further studies are needed to determine the molecular mechanism of the xanthone as a promising NO inhibitor.

PHOTOCHEMICAL SYNTHESIS OF γ-LACTONES FROM ALKENES

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The γ-lactone moiety is found in many natural products, products of biological importance, perfumes and food additives. For this reason, many synthetic approaches for their synthesis have been devised. Our group has already developed a photocatalytic protocol for the intermolecular synthesis of γ-lactones via photoredox catalysis.[1] Herein, we explore the possibility of employing small organic molecules as the photoinitiator to perform the same transformation. Lactones are obtained in good yield.

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DYNAMICS OF THE ANTIMICROBIAL EFFECT OF SUBSTITUTED N-PHENYL-1-HYDROXYNAPHTHALENE-2-CARBOXAMIDES

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Research and development of the new compounds with antimicrobial activity is still very necessary due to the antibiotic resistance emerging in many pathogens. Activity of the derivatives of *N*-phenylhydroxynaphthalenecarboxamides against methicillin-resistant *Staphylococcus aureus* (MRSA) has been found in previous studies. This contribution presents an evaluation of antistaphylococcal activity of *N* -phenyl-1-hydroxynaphthalene-2-carboxamides, see Figure 1, and its dynamics.

First screening of the activity of tested compounds was assessed by the microdilution method for detection of minimum inhibitory concentration (MIC). The most effective compounds with MIC in the range of 0.125 to 0.5 μg/ml, which is lower or comparable with the MIC of the reference antibiotic ciprofloxacin, were chosen for testing by the time-kill curves method. This method evaluates the antibacterial activity in dependence on time and concentration. The relationship between structure and activity was discussed.

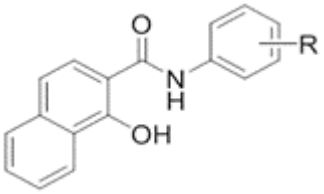

Figure 1: General structure of *N*-phenyl-1-hydroxynaphthalene-2-carboxamides.

DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF CHLOROACETYL DERIVATIVES OF 2-METHOXYESTRADIOL AND ABIRATERONE

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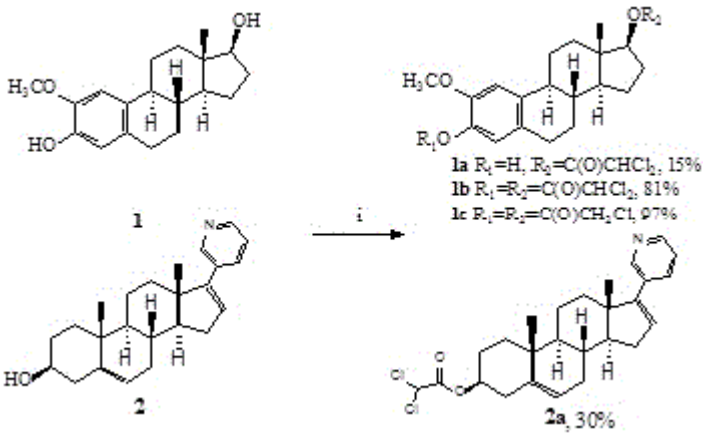
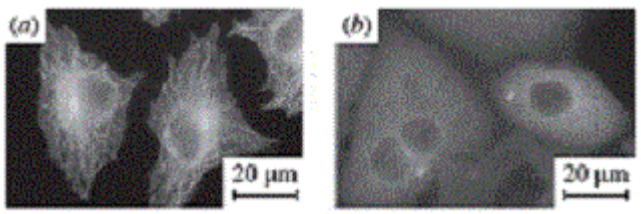
2-methoxyestradiol and abiraterone are known hormone-like compounds that are undergoing clinical trials and used in therapy of cancer. Also, dichloroacetate ion is known for its inhibitory ability towards pyruvate dehydrogenase kinase, which is also actively used in the therapy of malignant tumors. In the present study, we proposed an approach to the creation of dual drugs based on a dichloroacetyl fragment as an inhibitor of glycolysis and hormone-like compounds - 2-methoxyestradiol and abiraterone.



Reagents and conditions: i) $Cl_2CHC(O)Cl$ (for 1a, 1b and 2a) or $ClCH_2C(O)Cl$ (for 2c), 4-DMAP, CH_2Cl_2, STP, 24 hours.

For the obtained compounds, cytotoxicity was measured on the cell lines MCF-7 and HCT-116. It was shown [1] that the new derivatives have cytotoxicity in the micromolar range (EC_{50} = 1-50 μM, MCF-7), in some cases the activity is superior to the precursors. Immunofluorescence microscopy images were made of microtubules (MT) in human lung carcinoma A549 cells: (a) intact MT in 0.5% DMSO; (b) depolymerized MT in 10 mm solution of compound 1c.



Acknowledgments: This work was supported by the Russian Foundation for Basic Research (Project 20-03-00915A).

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DISCOVERY OF NEW ZIKA VIRUS NS5 PROTEIN INHIBITORS AS ANTI-ZIKV CANDIDATES BY VIRTUAL SCREENING BASED ON MOLECULAR DOCKING AND MACHINE LEARNING MODELS

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Zika virus (ZIKV) rapidly spread and caused a massive epidemic in 2016 in the Americas[1]. Despite causing mild symptoms, the major concern about ZIKV regards the severe neurological disorders, such as microcephaly and Guillain-Barré Syndrome[1–3]. ZIKV NS5 RNA dependent RNA polymerase (RdRP) and methyltransferase (MTase) are involved in viral replication, survival and immune system evasion[4], being important targets for the development of antivirals[1]. To date, there are no antivirals or vaccines approved to treat the infection, thus drug discovery against ZIKV is urgent. In this study, as part of the OpenZika project [5] supported by IBM's World Community Grid, we performed a virtual screening (VS) of the ChemBridge database against ZIKV NS5 RdRP and MTase, independently. The NS5 binding sites investigated were: RNA site, active and allosteric (NTP-site) for the RdRP domain, and SAH and GTP sites for the MTase domain[4]. The four filters used in VS were: (I) drug-like properties; (II) molecular docking in the respective sites; (III) phenotypic machine learning and Bayesian models for ZIKV and (IV) filtering for pharmacokinetic properties, PAINS and blood-brain barrier permeability. The VS prioritized 10 virtual hits that were experimentally tested in glioblastoma cells infected with ZIKV, through a High-Content Screening platform. In the cell-based assays, two compounds inhibited ZIKV replication, showing EC_{50} of 9.71 μM and 50.6 μM and low cytotoxicity (CC_{50} > 100 μM). Biophysical and enzymatic assays revealed that one compound inhibited NS5 RdRP with an IC_{50} of 5.04 ± 0.3 μM.

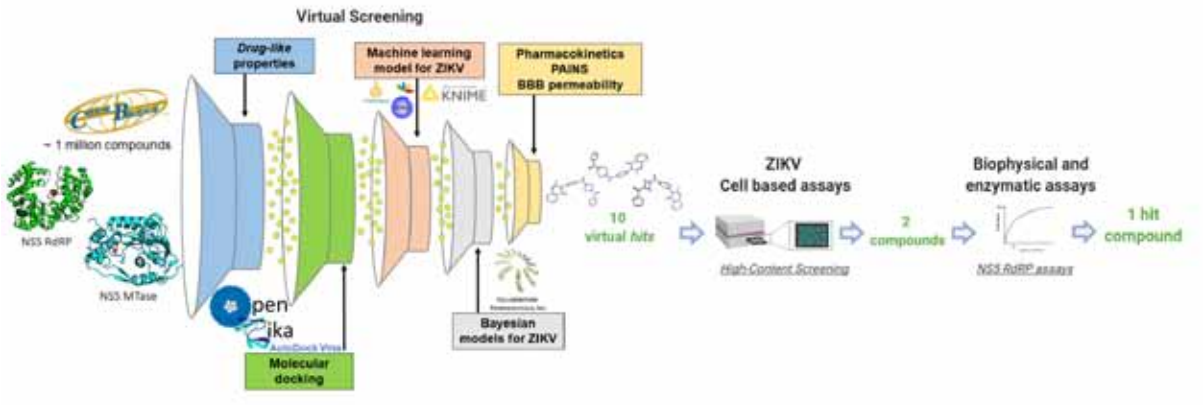

Figure 1. Workflow of virtual screening and biological evaluation in the search of new anti-ZIKV NS5 inhibitors. Created with BioRender.com

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SULFONAMIDE DERIVATIVES OF CIS-2,4,5-TRIS(ALKOXYARYL)-IMIDAZOLINES: DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION

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The preparation of cis-imidazoline derivatives from aromatic aldehydes with ammonia is a convenient and simple synthesis of the precursors of the known inhibitors Mdm2-p53 interaction [1]. Based on molecular docking (Figure 1), it was shown that sulfonamide derivatives of cis-2,4,5-tris (alkoxyaryl)-imidazolines can bind to the active site of the MDM2 protein.

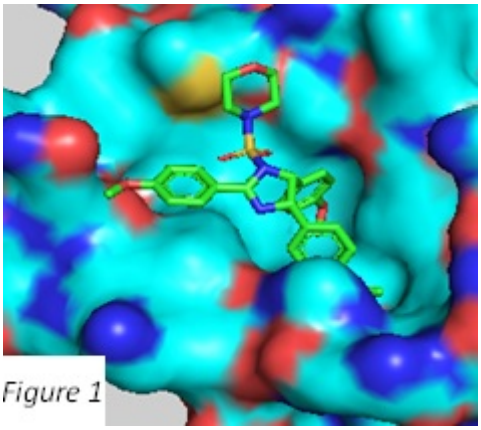
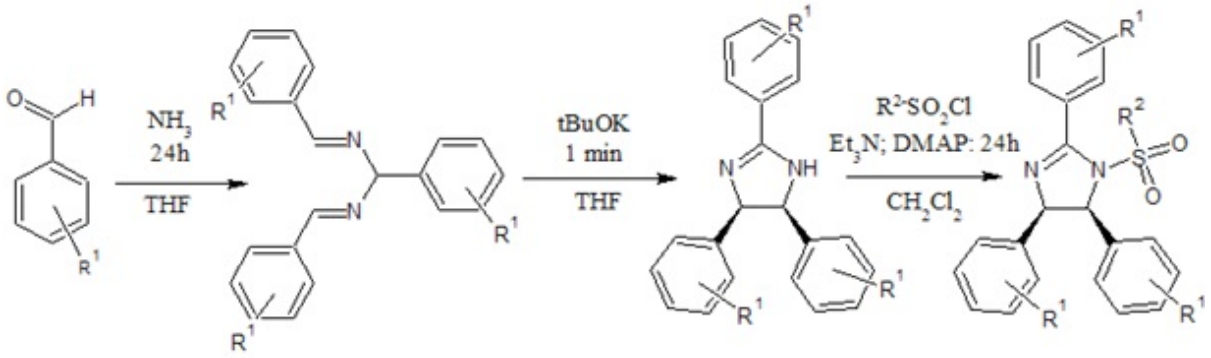


Figure 1

We report about novel series of imidazoline derivatives (synthetized by scheme 1) showed the ability to increase the level of p53, p21 and BAX proteins. Cytotoxicity of compounds in A549 cell lines is in micromole range.



Scheme 1. R_1=2,5-diMeO, 2,3-diMeO, 3,4-diMeO, 4-MeO, 2,4-diMeO. R_2=morfoline, piperidyl, pyrrolidinyl, Et_2N, 4-methylpiperidyl , Ts. Acknowledgments: This work was supported by the Russian Foundation for Basic Research (Project 20-03-00915A).

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DISCOVERY OF NEW CORRECTORS BASED ON NITROGEN HETEROCYCLIC SYSTEMS

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Background

Cystic fibrosis (CF) is a recessive genetic disease found primarily in Caucasians and caused by mutations in CFTR gene. Of the about 2000 known CF mutations, deletion of phenylalanine at position 508 (F508del) in the CFTR protein is the most common one.[1,2] F508del alters CFTR biosynthesis, resulting in a misfolded, rapidly degraded protein that is poorly trafficked out of the endoplasmic reticulum to the cell surface. Correctors are believed to specifically address the folding and trafficking defects of F508del-CFTR protein.[3] It is widely recognized that a combination of correctors of F508del-CFTR protein[4] with complementary mechanisms is desired.[5]

Essential methods

A library of compounds of our synthesis initially tested highlighted an hit scaffold, from which PP8 emerged as lead candidate to develop highly effective F508del correctors indicating a new complementary mechanism of action with class 1 correctors (VX-809). Having validated the mechanism of action of PP8 confirming its activity and synergy with VX-809, the chemical space of PP compounds was explored on SAR analysis to establish the critical structural requirements connected to the best corrector activity. Several rounds of synthesis highlighted some new potent analogues among which PP028, PP034, and PP037, with higher efficacy producing a rescue comparable to that of VX-809 and a strong synergism when used in combination with it.

The most potent compounds, were tested as correctors i) on primary airway epithelial cells (bronchial and/or nasal); ii) in biochemical assays and by microscopy to evaluate effect on F508del-CFTR maturation/trafficking. The pharmacological insight indicates that PP compounds possibly act as class 3 correctors (binding to NBD1 domain of CFTR).

Conclusions

Future objectives will be the evaluation of ADME properties of the best candidates to acquire information about drug-like properties. The obtained results will inspire the synthesis of new refined structures to further optimize efficacy and potency on F508del-CFTR and ADME properties.

After iteration of the process involving chemical synthesis and evaluation of the resulting compounds, we expect to achieve the best trade-off between corrector activity and drug-like properties with the aim of finding the best candidate for a possible preclinical development.

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NOVEL 2-AMINO-BENZIMIDAZOLE HYDRAZONES AS PROMISING ANTIPROLIFERATIVE AGENTS

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The structure of many clinically useful chemotherapeutic drugs is based on 2-aminobenzimidazolyl scaffold and its molucular modification allows generating new derivative with promising pharmacological activities [1]. Some of the 2-iminobenzimidazoles that have found application in the medicinal practice such as albendazole and flubendazole could inhibit hepatocellular carcinoma cell proliferation *in vivo* and *in vitro* as well as induced cell death in leukemia and myeloma cell lines. [2,3] Many other 2-aminobenzimidazole derivatives were synthesized and the biological screening *in vitro* showed that the studied compounds possessed relative high cytotoxicity against MDA-MB-231, HT-29, HeLa and Hep G2-cell lines [4].

The 2-aminobenzimidazole anthelmintic derivatives are able to block microtubule function in cells. As the key structural basis of microtubule, tubulin has become a highly attractive target for anticancer therapy. Several tubulin inhibitors have been approved as chemotherapeutic agents for different types of human cancers. Flubendazole has been shown to inhibite tubulin polymerization by binding tubulin at a site distinct from vinblastine [3].

In the present study, an evaluation of the cytotoxic activity of newly synthesized benzimidazole-2-yl-hydrazones was performed on MCF-7 (ER-positive breast adenocarcinoma) and AR-230 (chronic myeloid leukemia) cell lines. The synthesis of the hydrazones was accomplished by condensation of 1H-benzimidazol-2-yl hydrazine with benzaldehydes containing one or more methoxyl and one hydroxyl groups. The tested benzimidazole hydrazones showed high cytotoxic effect with IC50 values in the range 1.20 – 21.20 μM for the both cell lines. The 3,4,5-trimethoxyphenyl benzimidazole-2-yl hydrazone showed the most pronounced toxic effect on MCF-7 and AR-230 cells. In addition to the obtained promising cytotoxicity results, the effect of the studied compounds on the tubulin assembly was tested *in vitro* on purified bovine tubulin. The tested benzimidazole derivatives inhibited the tubulin polymerization.

Moreover, the anthelmintic activity of the tested compounds was evaluated *in vitro* against Trichinella spiralis demonstrating strong larvocide effect ranging from 100% to 90% at various concentrations.

Keywords: Benzimidazoles, anticancer activity, tubulin polymerization, *Trichinella spiralisl larvae*

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DITHIOCARBAMATES OF 9,10-ANTHRACENEDIONE DISRUPT TELOMERE FUNCTION BY TELOMERASE INHIBITION

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Telomerase is one of the hallmarks of cancer, important in tumor formation and progression and cellular immortalization. Given the up-regulated expression of telomerase is present in about 85% of all tumors, the enzyme is considered to be a desirable target for cancer therapeutics [1]. The main purpose of this work was to evaluate newly synthesized compounds as potential telomerase inhibitors.

Our results exhibit that compounds showed antiproliferative activity in non-small cell lung cancer at micromolar concentrations and inhibited colony-forming of cells in a short and long time treated. All tested derivatives inhibit telomerase activity in a concentration-dependent manner in the TRAP assay. Compounds show several cellular effects, including the ability to activate DNA double-strand breaks (DSB) via tumor lines that differ in telomere elongation mechanism. Dithiocarbamates activate DSB in the TERT-positive A-549 cell line, highlighted by the increased levels of γH2AX. This effect was not observed in treated TERT-negative HUVEC and NHBE cell lines and in the ALT-positive U2OS cell line, which indicates occurs of telomerase inhibition dependent induction of DSB. The crisis of cells was also attended with evidence of both senescence (SA-β-galactosidase activity), telomeres shortening, and apoptosis.

The results indicated that 9,10-anthracenediones represent an essential class of compounds for telomerase-related drug developments.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF FLUOROQUINOLONE-BASED QUATERNARY AMMONIUM ANTIBACTERIAL AGENTS

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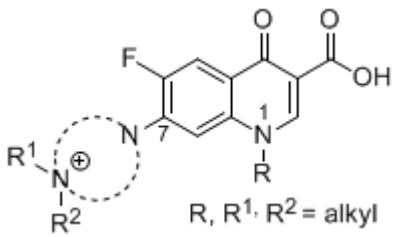
Quinolone chemotherapeutics target and inhibit the action of homologous type II topoisomerases, DNA gyrase and topoisomerase IV. Since the disclosure of nalidixic acid by George Lesher in 1962 (1) more than ten thousand congeners have been obtained from which four generations of bactericides with a broad spectrum of antibacterial activities have been developed (2).

Recently, we have synthesized a series of fluorescent fluoroquinolone hybrid compounds featuring fused quaternary quinolone-triazolinium moiety that exhibited biological effects (3). Novel derivatives showed *in vitro* antibacterial activity against various pathogens, including biofilm-forming *Pseudomonas aeruginosa*. The most active compounds were found to be comparable to the reference drug, ciprofloxacin and featured delayed antibiotic resistance development (3). The obtained conjugates were potent *E. coli* DNA gyrase inhibitors and caused a defect in DNA decatenation (3).

The aim of the present study was to obtain a series of novel fluoroquinolone-based quaternary ammonium compounds with pronounced antibiofilm activity. Moreover, the presence of quaternary nitrogen atom in the structure should prevent distribution to the brain and such hybrid agents should not elicit the direct CNS side effects after intravenous administration.

Fluoroquinolone derivatives bearing variety alkyl and amine substituents at N1 and C7 position of quinolone core, respectively, were design and synthesized. The obtained compounds were subsequently reacted with alkyl halides to give fluoroquinolones incorporating permanent positive charge on the nitrogen atom of the amine group. The products were characterized by NMR, IR, MS, X-ray crystallography and elemental analysis. Further, the obtained derivatives were evaluated for *in vitro* antimicrobial and antibiofilm activities against Gram-positive and Gram-negative biofilm-forming bacterial strains by means of total biomass and viability assessments (crystal violet and resazurin staining, respectively) in pre-exposure as well as post-exposure experiments. The most active antibacterial agents, ciprofloxacin analogues, exhibited antibacterial action in the low micromolar range, comparable to this of the original drug. Molecular docking experiments revealed that all the synthesized compounds were able to interact in the fluoroquinolone-binding mode at *Staphylococcus aureus* DNA gyrase and *Streptococcus pneumoniae* topoisomerase IV active sites. The selected compounds will be further subjected to extended studies. The planned tests are aimed at selecting optimized drug candidates that would exhibit enhanced biological activity, as well as low toxicity to minimize side effects.



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THROUGH REGIOSELECTIVE ORTHO-NITROANILINES REDUCTION TO BIOLOGICALLY ACTIVE HETEROCYCLIC COMPOUNDS

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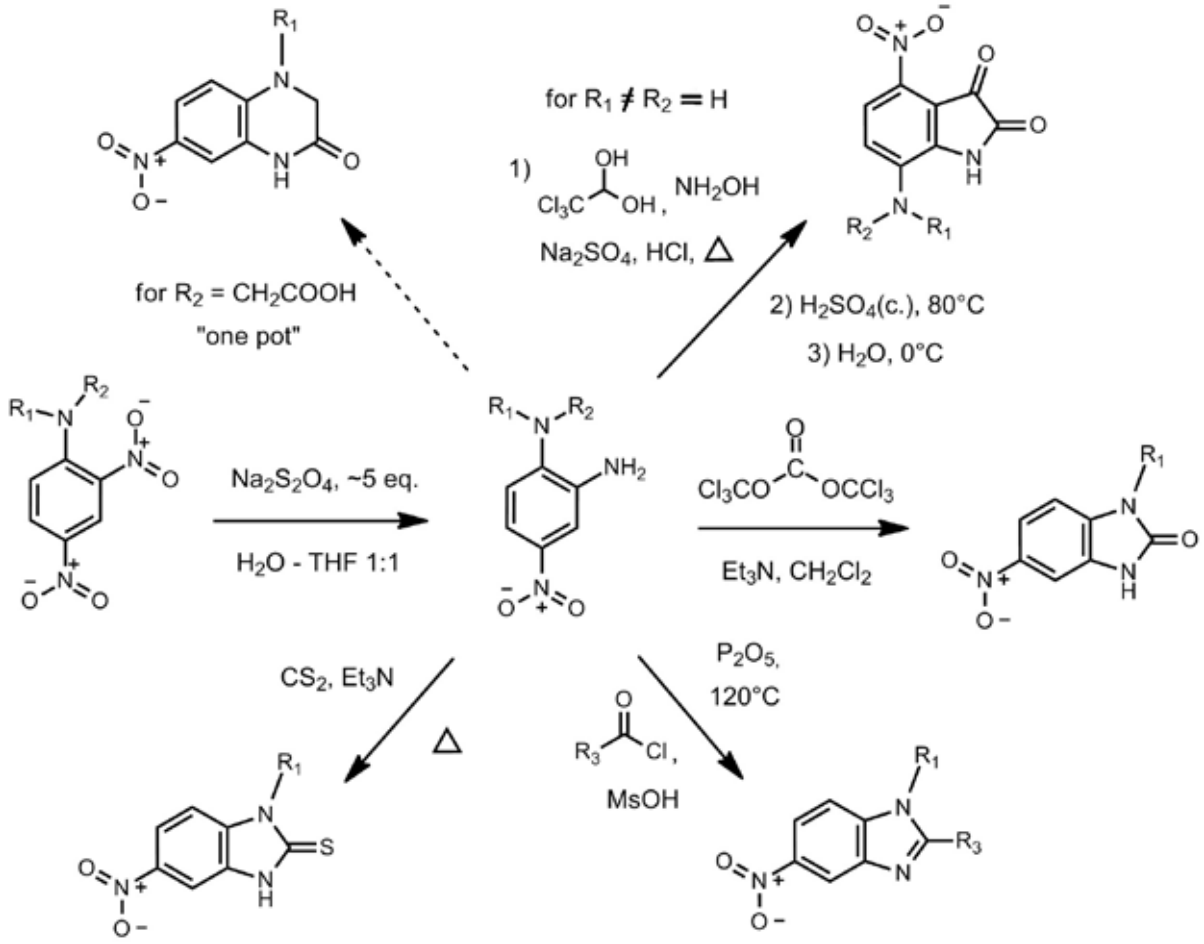
Currently, many types of drugs on the market have heterocycles in their structure. Our method allows the obtaining of different biologically active heterocycles from inexpensive and commercially available source – 2,4-dinitrochlorobenzene. Nucleophilic substitution of chlorine with nucleophilic amines followed by regioselective ortho-nitro group reduction lead to 2-amino-4-nitroanilines – useful building blocks for various heterocycles indicated in the scheme below. Thus, we can get scaffolds of biologically active compounds such as benzimidazolones/tions, benzimidazoles, and benzoxazoles (see scheme). In the last few years benzimidazoles and benzimidazolones have been studied extensively for their antitumor, antiviral and antimicrobial activities such as the antiprotozoal and antibacterial.

In this way, we proposed a simple method for design of new compounds with a wide spectrum of activity.



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STRUCTURAL CHARACTERIZATION AND STABILITY OF PROTEINS IN SOLID FORMS

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Protein therapeutics are becoming more and more important as an alternative treatment for a variety of diseases. For better stabilty, proteins are often formulated as solid pharmaceutical forms, the most common of which are lyophilized (freeze-dried) solid-powders. The stability of them is dependent on the preservation of protein native structure during lyophilization, as well as in the lyophilizates. During lyophilization cycle, proteins are exposed to several stress factors that, in combination with excipients can affect the protein strucutre in the final solid form. If the native structure of protein is not retained during lyophilization, this can be reflected in the unstable final pharmaceutical product, and thus in its quality, safety and efficiency. Characterization of proteins in solid is less established, as most analytical methods evaluate critical properties in solution, which is not necessarily an indication of adequate stabilization of the protein in the solid phase and thus the long-term stability of the pharmaceutical form. With characterization of proteins in solid, both secondary and tertiary structure can be evaluated during formulation development.[1,2] Only formulations that maintain structure in the solid state are then included in stability studies, which are of particular importance for the development of protein drugs. In this work we present the study of protein structure and stability in solid pharmaceutical forms using analytical methods such as FTIR, solid-state fluorescence, solid-state UV-Vis, , solid-state NMR and raman spectroscopy, as well as X-ray powder diffraction.

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NEW HETEROCYCLIC COMPOUNDS AS ANTI-INFECTIVE AGENTS AGAINST TRYPANOSOMIASES

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The need for new antitrypanosomal drugs continues to persist and aiming into this issue; we have prepared a large number of new heterocyclic compounds having different central rings [1,2]; that were further assayed against different trypanosoma subtypes. The present work describes the development and synthesis of 94 new heterocyclic compounds as agents against *Trypanosoma brucei* (*T. brucei*) and *Trypanosoma cruzi* (*T. cruzi*) infections. A relatively facile synthetic pathway has been amenable to a large number of functional groups, giving rise to a structurally diverse set of analogs. Among the most promising were the imidazopyridine, imidazopyrimidine, furopyridine and dihydroisoindol core rings. The set of compounds were designed using standard medicinal chemistry principles. We have modified eight portions of the central core based on specifical insertion of modifications at the positions **V**-**Z** and in regions **R^1**-**R^3**(**Figure 1**). Several compounds of this series exhibited an *in vitro* $EC_{50} \leq 1\mu M$ against *T. brucei* and *T. cruzi* parasites. All potent compounds were furthermore tested for toxicity against human lymphocytes CRL-8155, human hepatocytes HepG2, MRC-5 and PMM cell lines. The compounds exhibited no significant toxicity and high selectivity index (SI). The half-lives of four compounds were greater than 60 min, with a range of 84−100% of the test compounds remaining at the 60 min time point. The most active compound **1** arising from this series, also displayed the greatest plasma protein unbound fraction (FU = 9.9 %; EC_{50} value of 93.32 nM for *T. cruzi*; EC_{50} value of 18.12 nM for *T. brucei*). This compound was screened further in an acute model against *T.cruzi* TcTC2/*Tulahuen*. Overall, compound 1 (**Figure 1**) represents a potential lead for the development of novel drugs to treat Trypanosomiasis.

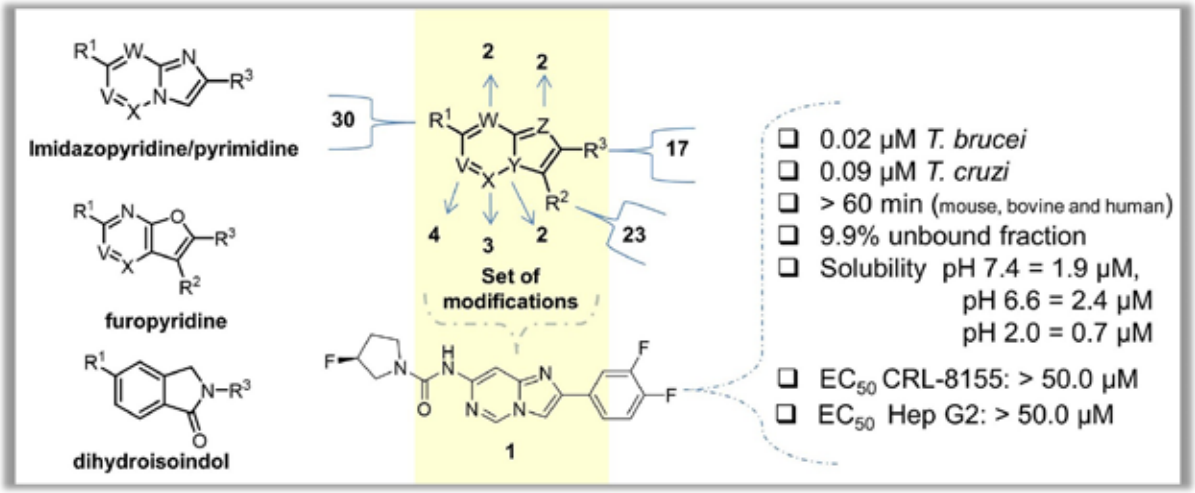

Figure 1. Set of modifications of the core ring. Antitrypanosomal activities, stability, protein binding, solubility and cytotoxicity results for compound **1**.

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PSYCHOTROPIC ACTIVITY OF A NOVEL ANILIDE-BASED 5-HT1A/5-HT7 RECEPTOR ANTAGONIST AND PDE4/7 INHIBITOR

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Cognitive and mood disorders are a growing health, social, and economic issue as they often co-occur and accompany various forms of dementia, depression, and schizophrenia. They are clinically relevant features in Alzheimer's disease which is one of the most prevalent age-related neurodegenerative diseases and whose treatment options are currently limited. There is an urgent need to develop novel drugs that will be able to effectively reduce cognitive and mood disorders. According to latest literature data, such multidirectional activity may be achieved by combining the interaction with serotonin 5-HT_{1A} and 5-HT_7 receptors and inhibition of cyclic-3′,5′-adenosine monophosphate (cAMP)-specific phosphodiesterase (PDE) 4 and 7 [1,2].

The aim of this study was to evaluate procognitive and antidepressant activity of selected 5-HT_{1A}/5-HT_7 receptor antagonist and PDE4/7 inhibitor using animal tests.

The procognitive and antidepressant activity was tested in Wistar rats using well-established experimental paradigms, *i.e.*, novel object recognition and forced swimming tests, respectively. Moreover, in order to exclude the possibility of competing behaviors such as general locomotor activity, the open field test was carried out and the influence of effective doses was studied.

Tested compound at a dose of 3 mg/kg (*i.p.*) significantly reversed MK-801-induced episodic memory deficits in the novel object recognition test, while at a dose of 10 mg/kg (*i.p.*) reduced the immobility time of animals (by about 34%) in the forced swimming test. The antidepressant-like effect produced by tested compound was stronger than that of escitalopram used as a reference drug. This study opens a new perspective in the search for an efficacious drug for the treatment of cognitive and mood disorders.

Acknowledgements:

This study was supported by the National Science Centre, Poland (Grant No. 2017/25/N/NZ7/00544).

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SYNTHESIS OF NEW LIGANDS OF THE QUINONE REDUCTASE 2 (MT3/QR2) BASED ON BENZIMIDAZOLONES

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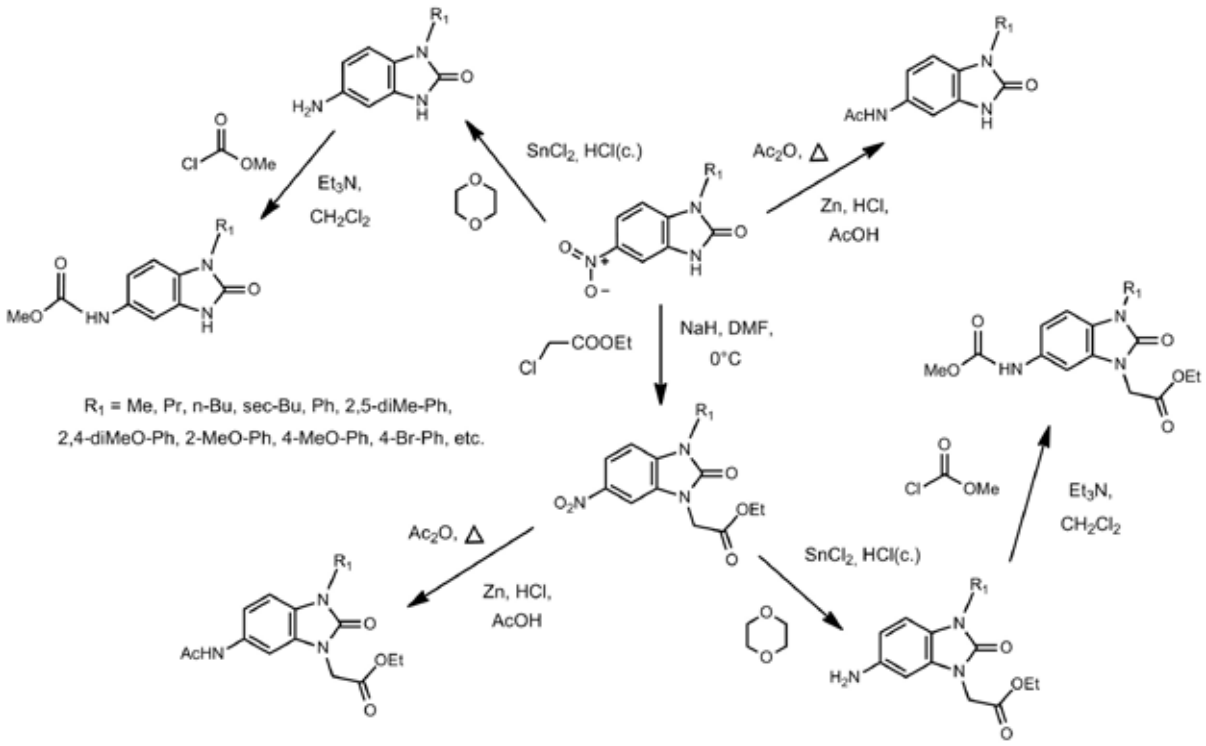
QR2 is a low-affinity melatonin receptor (MT3), that has neuroprotective, antioxidant, and IOP-decreasing properties. It is one of the perspective molecular targets for therapy of several diseases, such as glaucoma and Alzheimer's disease, that are of great social importance.

Recently it was shown, that 2-oxindole derivatives express noticeable MT3 activity, as an amide group is important for active site binding[a]. Therefore, it was reasonable to search for bioisosters with that fragment, as they have high chances to maintain the activity levels – so are benzimidazolones. The simple method of synthesis for 5-nitrobenzimidazolones was developed earlier in our group.

Further modifications include one-pot reduction and acylation of the nitrogroup in 6-position or 2-step synthesis of carbamoyl-derivatives. These substituents were chosen as they have shown the highest activity on 2-oxindoles. To increase the solubility of benzimidazolone derivatives in water, the nitrogen in 1-position was modified by a -CH_2COOEt fragment, as it can be hydrolyzed in the last step to obtain soluble salts, or by a -CH_2 CN fragment. These substituents were also rationally chosen, because 2-oxindole leaders have the -CH_2COOH or -CH_2CN group in 3-position.



This work was supported by the Russian Foundation for Basic Research (Project 20-03-00915A).

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NOVEL ANTITUMOR AGENTS BASED ON 3,7-DIAZABICYCLONONANE DERIVATIVES

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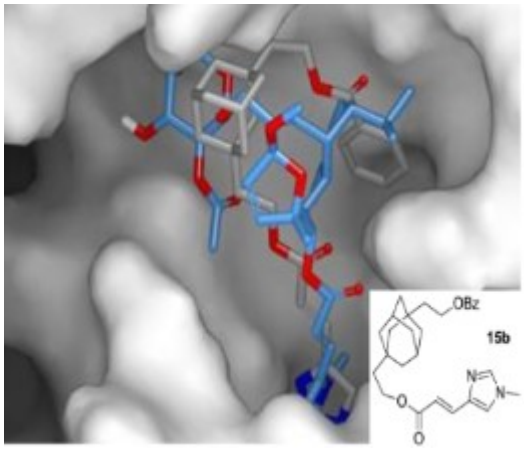
We constructed novel tubulin binding molecules using a simple polycyclic framework as a central scaffold that directs the substituents into the key binding pockets of the molecular target[1]. In this work we have chosen 3,7-diazabicyclonane core as the central block with *meta*-substituted aryl groups in the bridgehead positions and some hydrophilic substituents (such as succinic acid moiety) in the positions 3 and 7.



We synthesized derivatives of 3,7-diazaadamantane from *m*-xylene and *m*-fluorotoluene by radical bromination followed by nucleophilic substitution of bromine to cyanide, acidic hydrolysis, acid ketonization and Mannich reaction.[2]Target molecules were obtained by aminal degradation with an acyl chloride (Fig. 1). The cytotoxicity of obtained compounds were investigated in different cancer cell lines.

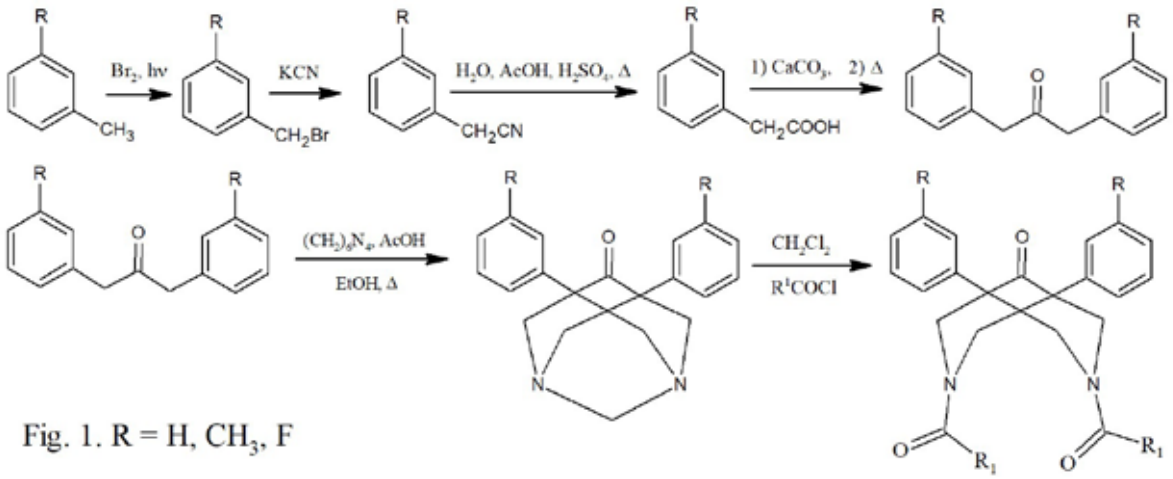


Fig. 1. R = H, CH_3, F

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This work was supported by the Russian Foundation for Basic Research (Project 20-03-00915A)

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NOT ONLY AFFINITY - MACHINE-LEARNING-BASED TOOLS FOR OPTIMIZATION OF LIGAND PHYSICOCHEMICAL PROPERTIES

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A broad range of computational strategies that are applied at every stage of drug design process is aimed to minimize its time and costs and maximize its effectiveness at the same time. Various methods help in the identification of new potentially active compounds (ligands), optimization of their physicochemical and pharmacokinetic profiles, but the important step of data management at every stage of drug design pipeline (even after introduction of the drug to the market, e.g. during monitoring the side effects) also cannot be neglected.

Due to the increasing amount of data, both in the *ligand-* and *structure-based* fields, the power of simple statistical methods is often not sufficient to provide proper discriminants between active and inactive compounds. In this era of exponential growth of the amount of information (not only in the field of chemistry and pharmacy), machine learning (ML) methods have become extremely important and useful tools. They are able to deal with large-scale, and high-dimensional data, and therefore their application in various fields is becoming wider and wider.

Currently, a number of approaches for prediction of ADMET properties are available. They are mostly ligand-based tools and two classes of models are constructed – classification ones (mutagenesis/non-mutagenesis, stable/unstable, soluble/insoluble, etc.) or regression tools are applied and QSAR-type models are formed, in which quantitative impact of particular structural moieties on considered parameters is examined.

The current project is composed of two main parts: construction of a ML-based tool for evaluation of physicochemical and ADMET properties of compounds and development of methodology for optimization of chemical structures (in terms of physicochemical and ADMET properties) on the basis of the constructed predictive models. The set of evaluated properties include: solubility, metabolic stability, biological membranes permeability, hERG channels blocking, and mutagenicity.

Analysis of training sets (prepared on the basis of ChEMBL data) was performed with the use of the classification and regression ML algorithms. It predicts the evaluated parameter for submitted compounds and automatically generates the structurally related ligands, providing analogous evaluations as for the input structure. Such an approach allows not only to assess compound physicochemical properties, but also to simultaneously optimize its structure in terms of evaluated property.

Acknowledgments

The study was supported by the grant OPUS 2018/31/B/NZ2/00165 financed by the National Science Centre, Poland (www.ncn.gov.pl).

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SYNTHESIS AND MOLECULAR MODELING OF SOME CATECHOL DERIVATIVES AS ACETYLCHOLINESTERASE INHIBITORS

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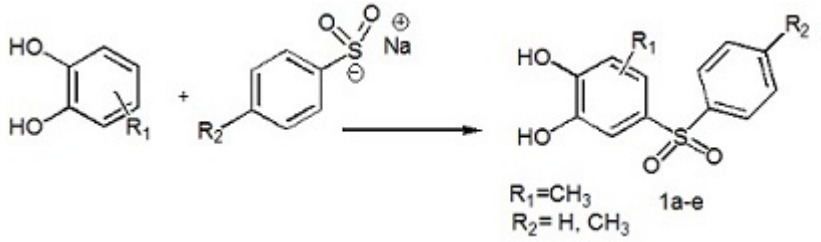
Alzheimer's disease (AD) is one of the neurodegenerative disorders. Acetylcholinesterase inhibitors are used in treatment of this disease. In recent years, many studies have been performed to design and develop new acetylcholinesterase inhibitors (1).

In this study a group of catechol derivatives were synthesized by electrochemical method as acetylcholinesterase inhibitor. Catechol was oxidized electrochemically by applying appropriate voltage in the presence of different nucleophiles (benzenesulfinate derivatives) (2,3). The obtained compounds were filtered and washed by water. Molecular docking study of designed compounds was performed against acetylcholinesterase (PDB: 1EVE) by Autodock Vina to find the main interactions and binding mode.



All the compounds were synthesized in a good yield and purity. The structure of obtained compounds was characterized by ^{1}HNMR and LC-MS. According to the docking study most of the compounds are able to form π-π interactions with Trp84 and Phe330. The compounds with appropriate interactions were selected for further in-vitro and in-vivo investigations.

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MOLECULAR DOCKING OF NATURAL PRODUCTS AS ANTIOXIDANT AGENTS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Molecular docking is an intensive and prominent computational method in the drug discovery process. The benefit of docking is to identify how the ligands bind at the enzyme or receptor binding site through specific key interactions and to predict the binding affinity between protein-ligand complexes (GUPTA; MOHAN, 2014). In this study, the crystallographic structures of the enzymes acetylcholinesterase (AChE) (PDB: ID 4EY6) and nitric oxide synthase (NOS) (PDB: ID 1NSI) were selected in complex with their respective ligands, obtained from the Protein Data Bank (PDB) in the Research Collaboratory for Structural Bioinformatics (BERMAN et al., 2000), later the structures were prepared for the experiment by adding hydrogen atoms and removing water molecules and inhibitors. Soon after, the Gold 5.8.1 software was used to investigate the modes of interactions between molecules and generate a set of conformations for these ligands. The molecules selected for the study were as follows: asiaticoside, curcumin, demethoxycurcumin, bis-demethoxycurcumin, calebin A, ginkgolide A, ginkgolide B, ginkgolide C, bilobalide, honokiol, linalool, carvacrol, luteolin, rosmarinic acid, geraniol, dihydrotanshinone, tanshinone I, methylenetanshinone, cryptotanshinone, salvianolic acid A, salvianolic acid B, rosmariquinone and quercetin. In this study, the following results were obtained: in the interactions between the molecules and the amino acid residues of the AChE catalytic site, it was observed that Trp86 was the amino acid that obtained the highest affinity, followed by the amino acid Tyr124, thus the molecules that obtained the best results for the target were demethoxycurcumin, bis-demethoxycurcumin, calebin-A, ginkgolide A, ginkgolide B and ginkgolide C, linalool, carvacrol, luteolin, dihydrotanshinone, tanshinone I, cryptotanshinone, methanolic acid and rosanquinone and physostigmine. In the docking simulation with the NOS enzyme, it was observed that there were only hydrogen interactions, where the amino acid Gln 263 was the one that obtained the greatest interaction with the catalytic site of the enzyme, followed by the amino acids Glu377 and Arg388, the molecules that presented the best results for the NOS target were curcumin, calebin-A, ginkgolide A, ginkgolide B, ginkgolide C and salvianolic acid B, asiaticoside and salvianolic acid A. Thus, it was possible to conclude that the studied molecules made interactions with the AChE and NOS enzymes, which demonstrates that these ligands can be promising candidates for laboratory tests.

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SYNTHESIS, MOLECULAR DOCKING AND LIPOPHILICITY ESTIMATION OF NOVEL ANTIPROLIFERATIVE POLYHALOGENATED BENZOTRIAZOLES AS CASEIN KINASE INHIBITORS

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Tetrabromobenzotriazole (TBBT) is a selective inhibitor of human CK2 enzyme at a submicromolar concentration. We have developped new TBBT derivatives incorporating a hydrophilic/hydrophobic groups at N1 or N2 of benzotriazole nucleus. All compounds showed inhibitory activity to ck2 at low submicromolar concentrations with N2 regioisomers exhibited higher antiproliferative activity against breast and lung cancer cell lines (MCF-7, and A549, respectively) in a lower micromolar concentrations than N1 isomers. Our docking studies illustrated that all compounds occupies the ATP binding site similar to the parent TBBt. Hydrophilic groups at N2 of the TBBt triazole nucleus provides binding with important residues like Asp175, Lys68 and Trp176. Changing the position and nature of substituting group alters the binding mode and hence affects ck2 activity. Herein, we reported the experimental measurement of the lipophilicity for TBBt derivatives for the first time. Our study demonstrated that TBBt hydrazides are more lipophilic than their corresponding acids which is opposing the lipophilicity calculated by Molsoft, MOE, ChemDraw and ALOGPS 2.1. This may explain the greater anticancer activity of hydrazides over their corresponding acids despite their nearly equipotent enzyme inhibition.

MOLECULAR DOCKING STUDIES OF XANTHONE DERIVATIVES FROM THE EPICARP OF GARCINIA MANGOSTANA (CLUSIACEAE) TO INCREASE THE INHIBITORY ACTIVITY AGAINST DIGESTIVE ENZYMES

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Abstract:

Enzymes such as α-amylase and pancreatic lipase are important digestive enzymes in the metabolism of lipids and carbohydrates, being them attractive therapeutic targets for the treatment of obesity and type 2 diabetes [1-3]. Prenylated xanthones with promising inhibitory activity on α-glucosidase have been isolated from garcinia *Garcinia mangostana*, popularly known for its edible fruit (mangosteen), and through computational studies more active compounds have been designed and synthesized from xanthones [2,4]. This shows the potential of xanthones from *G. mangostana* as inhibitors of digestive enzymes related to obesity and type 2 diabetes. In the present investigation, a pharmacodynamic optimization study is carried out to enhance the inhibitory action against α-amylase and pancreatic lipase of some xanthones obtained from the epicarp of *G. mangostana* fruit based on computational methodologies. The methodology included the isolation and identification of bioactive xanthones from the epicarp of *G. mangostana* fruits. Subsequently, a pharmacodynamic optimization study was conducted to design potentially more active naturally-inspired molecules. In the computational study, the site and mode of interaction against each enzyme, α-amylase and pancreatic lipase were established, these enzymes were obtained from PDB with code: 4GQR and 1LPB, they were downloaded and prepared for docking molecular studies, said coupling was carried out with the Autodock4, Autodock Vina and Glida programs, the latter in order to obtain more reliable results, that said, the optimization carried out was established from xanthones with activity, said design was carried out in terms of improving their potential for simultaneously inhibit enzymes. The chemical study allowed the isolation and identification of five xanthones, being α-Mangostin and γ-Mangostin the most active compounds. From the computational study, initially pockets were established in which the catalytic interruption of the enzyme was being generated, from there the pharmacodynamic optimization process began in which structural modifications were established on the phenolic groups that would increase their affinity for the enzymes, using for this compounds with groups acceptors and donors of hydrogen bonds, obtaining five derived molecules formed by poly-oxygenated benzoate esters, with better interactions against enzyme targets. These molecules increased their molecular coupling energy, their modes of interaction, as well as the amount of interaction residues in the protein ligand complex.

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STRUCTURAL STUDY OF ANTIPOLYMERIZATION PEPTIDE EXTRACTED FROM PELOID

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Studies devoted to the research of biologically active compounds obtained from plants occupy one of the leading places in the search for new drug candidates. The ability to extract ready-to-pharmacological evaluation or easily modified highly effective substances to achieve clinically significant results prompts large-scale screening studies and the identification of plant groups that are potential "suppliers" of biologically active molecules. Peptide compounds are attracting special attention, since the relative simplicity of their structure and wide possibilities for chemical modifications make it possible to count on the creation of new pharmacological preparations. Peptides with anticoagulant, antimicrobial, membrane-stabilizing, antioxidant activity are being intensively studied. Earlier it was shown that glyprolines Pro-Gly, Pro-Gly-Pro, Gly-Pro demonstrate anticoagulant, fibrin-depolymerization and antifibrin-stabilizing activity[1]. Some studies have shown that short-chain peptides can simultaneously demonstrate multiple biological effects, affecting several enzymatic, transport, or receptor systems simultaneously[2].

It is of theoretical and practical interest to study the structure, spectrum and mechanisms of the biological effects of the antipolymerization peptide compound studied in the laboratories of the Tyumen State Medical University, which was isolated and purified from peloid[3]. In the course of the work, the primary structure of this peptide was first identified. For the first time, the spatial structure of the peptide was modeled, the functionally significant areas in the structure were identified, and the origin was established using in silico methods.

It was shown in the experiment that the isolated peptide increases the time of fibrin clot formation both in vitro and in vivo. The state of coagulation and microcirculation in laboratory animals was investigated against the background of the effector administration. Observation results show that biological effects are not limited only to interactions with coagulation factors or platelet receptors, but also to changes in leukocyte reactions, rheological characteristics of blood flow in case of endothelial damage.

The results obtained make it possible to assess the prospects for further pharmacological study of antipolymerization from sapropel according to the scheme provided for preclinical tests, and can be used to develop new means of pharmacological correction of hemostasis.

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ASSESSING ANTICOAGULANT EFFECT OF NEW PHARMACOLOGICAL COMPOUNDS

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Pharmacological agents providing controlled regulation of the activity of the hemostasis system are one of the most demanded groups of drugs. This is due to the high frequency of thrombohemorrhagic complications accompanying a wide range of pathological conditions, including cardiovascular, infectious and other diseases. The study of plants as sources of compounds with the ability to inhibit fibrin polymerization, and as a consequence, thrombus formation, is one of the areas of research carried out in the laboratories of the Tyumen State Medical University for a long time. In the course of the research, the presence of peptide compounds in a number of plant families growing in Western Siberia was proved, which clearly and dose-dependently inhibit the formation of polymer fibrin in in vitro and in vivo experiments. The possibility of creating pharmacological agents on the basis of the studied biologically active substances prompts a detailed study of the structural and functional characteristics of plant peptides.

In the present study, the effect of peptide anticoagulants obtained from various natural sources was assessed using such coagulation tests as: activated partial thromboplastin time; prothrombin time; thrombin time; tests characterizing the activity of the fibrinolytic system, the formation and level of fibrin monomers; tests characterizing the autopolymerization of fibrin monomers; tests characterizing the activation of fibrinogen by proteases in the presence of test compounds. The selected combination of routine clotting tests, widely used in clinical practice, and instrumental tests designed to solve scientific research problems, helps at the stage of laboratory assessment of a new biologically active compound or pharmacological agent to detail the mechanism of action of the effector on the blood coagulation system, to establish the points of application of the realized effect , which will subsequently make it possible to determine the spectrum of pathological conditions for which the target compound will be most effective and to conduct further preclinical and clinical studies.

NEW POTENT BENZISOXAZOLE DERIVATIVES AS PLEIOTROPIC COMPOUNDS WITH 5HT4R AGONISM AND IN CELLULO ANTIOXIDANT AND NEUROPROTECTIVE PROPERTIES AGAINST ALZHEIMER DISEASE

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In a world where life expectancy is increasing, Alzheimer disease (AD) is the main cause of dementia in the world. This is a progressive neurodegenerative disorder characterized by memory loss and cognitive decline. Despite the fact that the physiopathology of AD is not entirely known at the time, some molecular causes were found such as the ß-amyloid peptides aggregation, tau-dependent neurofibrillary tangles, as well as oxidative stress and neuroinflammation. Currently, treatments available for patients are mainly acetylcholine esterase (AChE) inhibitor, which only have symptomatic benefits and do not cure AD. The medical need is thus strong in AD population.

In this context, the concept of Multi-Target Directed Ligands (MTDLs) was applied to design a drug with several therapeutic targets. The envisaged MTDL (Targeted structure – fig 1) should be able in first hand, to limit the development of ß-amyloid plaques obtained by the aggregation of ß-amyloid peptides (Aß). Indeed, our compounds are designed to promote the cleavage of amyloid protein precursor (APP) by α -secretase activation in order to produce a neuroprotective and soluble peptide sAPPα. This is the role of the $5HT_4R$ agonists which are already studied in the CERMN in other MTDL projects and led to the discovery of Donecopride[1] (blue part – fig 1.). In another hand, it appears that the oxidative stress plays a central role in AD.[2] Adding antioxidant moiety such as polyphenol, lipoic and ferulic acid (red part- fig 1.) could trap free radicals or reactive oxygen species (ROS) and also have a neuroprotective effect. This aspect has been widely studied in Prof. Maria-Laura Bolognesi's laboratory over the years.[3] To that end, different compounds will be designed and synthesized, with the expertise of CERMN and Prof Maria-Laura Bolognesi, in order to evaluate theirs in vitro/in vivo properties regarding their agonist activity on $5\text{-}HT_4R$ and antioxidant properties. The development and promising in vitro/in cellulo results of the benzisoxazoles's moieties lines will be described in this poster.

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CONJUGATION WITH THE CELL PENETRATING PEPTIDE ANGIOPEP-2 ALLOWS BRAIN DELIVERY OF A MITOCHONDRIOTROPIC INHIBITOR OF POTASSIUM CHANNEL Kv1.3

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The voltage-gated potassium channels $K_v1.3$ located in the inner mitochondrial membrane is highly expressed in several cancers such as melanoma, pancreatic cancer (PDAC), glioblastoma and neuroblastoma, exerting important roles. Inhibition of mito$K_v1.3$ leads to a cascade of events, ultimately leading to pro-apoptotic processes, unlocking the possibility to the design of selective inhibitors[1]. PAPTP, one of these novel inhibitors of mito$K_v1.3$, drastically reduces tumor growth in mouse models of melanoma and PDAC, without severe side effects [2]. Unfortunately, despite being effective against glioma cells *in* vitro, PAPTP is not able to cross the blood brain barrier (BBB), resulting completely inactive *in vivo*[3].

One of the approaches exploited to overcome the BBB is based on cell-penetrating peptides (CPPs), short sequences of amino acids capable of crossing biological membranes and of delivering active drugs. Angiopep2 (TFFYGGSRGKRNNFKTEEY) and TAT (GRKKRRQRRRPPQG) are among the most studied for brain delivery[4-6].

The aim of this study is to synthesize Angiopep2-PAPTP and TAT-PAPTP and to evaluate their absorption into the brain *in vivo*.

The conjugates were designed using the pro-drug approach. To obtain this, the TPP^+ moiety of PAPTP was modified adding a linker to one of the phenyl groups (PAPTPL) and then conjugated to the CPPs through a bio-reversible carbamate bond.

TAT-PAPTP and Angiopep2-PAPTP were both tested *in vivo*. TAT-PAPTP was abandoned because of toxicity signs. Angiopep2-PAPTP was administered to C57CL/6 mice (5 μmol/kg b.w.), which were sacrificed after 15 (n=5), 30 (n=4) and 60 (n=6) minutes.

The results show that Angiopep2-PAPTP is present in the brain at 15 and 30 minutes after the injection. The analysis of the liver showed that Angiopep2-PAPTP is mainly metabolized through the cleavage of the peptide chain. In particular, PAPTPL plus the first one, two or three amino acids of the chain were identified via HPLC/MS analysis.

Summarizing, while PAPTP itself is not able to reach the brain, its conjugation with Angiopep2 represents a promising strategy to overcome the BBB.

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DEVELOPMENT OF INTRANASAL PEPTIDE NANOVACCINE AGAINST GROUP A STREPTOCOCCUS

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Vaccination is the cost-effective approach to prevent infectious diseases. Weakened or dead whole organism-based vaccines may be associated with the risks of allergic responses. Peptide-based vaccines possess greater safety profile compared to whole organism vaccines due to exclusion of unwanted biological material and ease of synthesizing it in highly pure state and large scale. However, peptide-based vaccines are poorly immunogenic alone and demand an addition of immunoadjuvants. In our lab, we developed a novel self-adjuvanting delivery system to improve the immunogenicity of group A streptococcus (GAS) peptide-based vaccines. We conjugated anionic polymer polyglutamic acid to a peptide comprising of GAS J8 B cell epitope and universal T-helper epitope. The peptide-polyglutamic acid conjugates were further formulated into nanoparticles by complexation with cationic trimethyl chitosan (TMC). The mice vaccinated with this nanovaccine generated higher serum antibody titers compared to mice receiving peptide admixed with cholera toxin B mucosal adjuvant (positive control). We further optimized this nanovaccine by incorporating lipid moiety and established structure activity relationships of nanovaccines by varying spatial arrangements of lipid and T-helper epitope. The combination of lipid and nanoparticles had a synergistic effect on the stimulation of immune responses against peptide antigens. We also found out the optimum length of polyglutamic acid into the conjugates and used more cationic fungal TMC into the formulation to check its effect on the immunogenicity of nanovaccines. Thus, we developed peptide-based nanovaccines by inducing desired charge (negative) on the antigenic peptide through conjugation strategy and formulating the conjugate with the oppositely charged (positive) polymer through complexation. This self-adjuvanting delivery system may serve as a choice for the design of peptide-based vaccines against variety of pathogens.

4,4-BIS(3-METHYLTHIOPHEN-2-YL)BUT-3-EN-1-YL DERIVATIVES OF N-BENZYLAMIDES AS MGAT3/4 INHIBITORS WITH ANALGESIC ACTIVITY

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Proper functioning of the central nervous system (CNS) depends, among other aspects, on neurotransmission of γ-aminobutyric acid (GABA). GABA transporters (mGAT1-4) belong to the solute carrier 6 (SLC6) gene family and are responsible for GABA reuptake. Inhibition of mGAT1-4 is exploited in search for the treatment of several neurological disorders including epilepsy[1] and neuropathic pain (NP).[2] mGAT1, which is a biological target of tiagabine, an antiepileptic agent, is currently best described. Therefore, understanding pharmacology of other GATs could help to clarify their role in the CNS.

In this study, potent mGAT3/mGAT4 inhibitors, 2-substituted derivatives of the *N*-benzylamides of 4-hydroxybutanoic, 4-hydroxypentanoic, 4-aminobutanoic acid, and serine analogs are presented. Special attention is paid to their potential application in the treatment of neurological disorders with an emphasis on analgesic activity in the neuropathic pain model. The inhibitory potencies of the presented compounds were determined for the four mouse GABA transporter subtypes (mGAT1 to mGAT4) through [^{3}H]GABA uptake using human embryonic kidney cells (HEK-293) stably expressing the mouse GABA transporters.[3] Two selected hits (**1** and **2**, Fig.1) showed a statistically significant antiallodynic activity in the von Frey test in diabetic and oxaliplatin-induced neuropathic pain model.

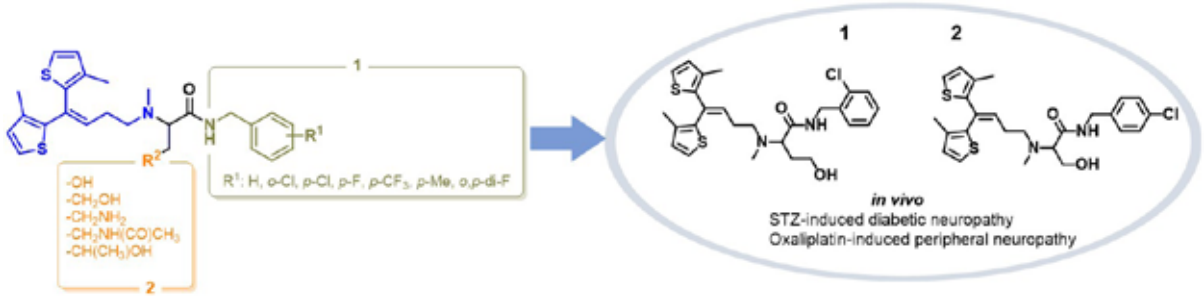

Figure 1. The general structure of target compounds.

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ADSORPTION OF INHIBITORS TO THE CRYSTAL SURFACE OF HAEMOZOIN: A TOOL FOR THE RATIONAL DESIGN OF NOVEL ANTIMALARIAL DRUG CANDIDATES

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The malaria parasite produces malaria pigment (haemozoin) as the product of the haem detoxification process following haemoglobin degradation.[1-3] Haemozoin is a crystalline material which comprises of μ-propionato ferrihaem dimers which are hydrogen bonded *via* propionic acid side chains.[4] Quinoline antimalarial drugs, including chloroquine and quinine, have been shown to inhibit the crystallization process. However, this class of drugs has been severely compromised by resistance, [5] highlighting the urgent need for new antimalarials.

It has been proposed that antimalarial drugs inhibit the formation of β-haematin (synthetic haemozoin) via adsorption to the fastest-growing crystal face(s). Accelrys Material Studio has been used to investigate crystal growth morphology, as well as the interactions between known inhibitors and the four major crystal faces. The adsorption energies of a wide variety of cyclic scaffolds, including their relevant protonated forms at pH 4.8, have also been determined. Notably, the quinoline nucleus present in chloroquine and quinine, as well as the acridine nucleus present in quinacrine, show favourable adsorption to the fastest-growing {001} face. The adsorption of inhibitors onto the {001} face has proven to be an adequate computational model to predict the inhibition of β-haematin formation. The phenoxazine scaffold has been identified as a strong adsorber and number of model compounds based on this scaffold were investigated computationally. These compounds have since been synthesised and show good β-haematin inhibition activity.

The computational tool developed in-house has facilitated the design of active β-haematin inhibitors, which shows promise for the development of new lead compounds.

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NOVEL ACRIDINE DERIVATIVES AS TDP 1 AND/OR 2 INHIBITORS

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Cancer is a one of the most deadly diseases, responsible for about 13% of all deaths worldwide. It is normally caused by genetic abnormalities related to DNA of the affected cells, therefore inhibition of DNA repair enzymes can induce DNA damage leading to cell death. Among the most recently discovered DNA repair enzymes are Tyrosyl-DNA phosphodiesterases TDP1 and 2, which function is excising irreversible protein tyrosyl-DNA complexes involving topoisomerase 1 and/or 2-DNA complexes. TDP1 catalyzes the hydrolysis of the phosphodiester bond between Top1 and DNA-3′-phosphate, suggesting a role in repairing of DNA double-strand breaks. Additionally, TDP2 removes many covalent adducts from DNA through hydrolysis of complexes between DNA and the Top2 active site tyrosine residue. TDP inhibitors reduce the destabilization and cleavage of these complexes, making them irreversible and thereby driving cancer cells into apoptosis[1].

Here, we describe the design, synthesis and pharmacological evaluation of novel amino substitutent tricyclic analogues as TDP1 and/or 2 inhibitors. The new compounds bear the aza-acridine core, possessing one or two basic side chains in the presence or not of a methoxy group.

All compounds were tested for their activity against TDP1 and 2 and among them disubstitutent derivatives showed significant activity.

The early results showed in accordance with *In Silico* calculations, that the second basic side chain is essential for the activity against TDP1 and 2. Additionally, crucial for this activity is the precence of the methoxy group.

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PHARMACOPHORE-BASED INSIGHT INTO STRUCTURAL DETERMINANTS FOR HIGH 5-HT6R AFFINITY AMONG ORIGINAL TRIAZINE DERIVATIVES WITH POTENTIAL PROCOGNITIVE ACTIVITY

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Serotonin 5-HT_6 receptors have been a popular protein target for over 20 years in the search for new therapeutic agents for the treatment of diseases and dysfunctions of the central nervous system, including depression, Alzheimer's disease, schizophrenia or obesity [1]. However, none from the 5-HT_6R agents have reached pharmaceutical market yet, thus searching for the structurally novel, highly active 5-HT_6R ligands with desired pharmacokinetic profile is both challenging and necessary field of drug development research.

The subject of the presented research is a series of 21 novel triazine-based derivatives **(1-21)**: unsubstituted **(1)** and substituted with two chlorine **(2-19)** or fluorine atoms **(20,21)** in the aromatic ring **(R^1)** and containing different length **(n)** and branching **(R^2)** of the linker (Fig. 1).

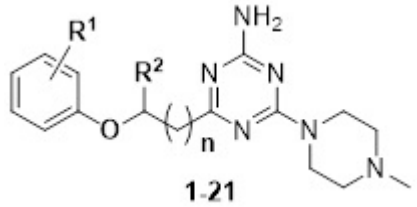

Fig. 1. General structure for investigated compounds.

Among the investigated series, nine derivatives showed very high affinity with K_i < 20 nM. Additionally, one of the most active compounds (K_i= 6 nM) displayed subnanomolar antagonistic action (K_b = 27 pM) and procognitive effect *in vivo* in Novel Object Recognition (NOR) test in rats [2].

The structures of the triazine-based derivatives match quite well to triangle topology characteristic for 5-HT_6R antagonists [3], despite the fact that this class of compounds does not contain sulfone nor indole moiety, as majority of 5-HT_6R ligands [4]. Pharmacophore-based analysis, performed within this study led to the highlighting the differences of presented derivatives from other reported 5-HT_6R ligands and to determine structural factors which provide such high activity among this original chemical class of compounds.

The project is financed by the National Science Center grant No. NCN 2018/31 / B / NZ7 / 02160. Some of the syntheses were made as part of the Students' Group of Medical Chemistry.

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SPLICE-SWITCHING SMALL MOLECULES AS INDUCERS OF APOPTOSIS

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The Bcl-2 protein family are essential gatekeeper regulators of apoptosis.1 One such protein, Bcl-x is of particular interest as a therapeutic target. It has two splicing isoforms; pro-apoptotic Bcl-xS and the anti-apoptotic Bcl-xL; the latter of which is upregulated in a variety of cancers. Thus, exogenous regulation of Bcl-x splicing, which biases the pathway towards the pro-apoptotic Bcl-xS isoform could provide a new a novel mechanism for
cancer therapy.2,3 (Figure 1)

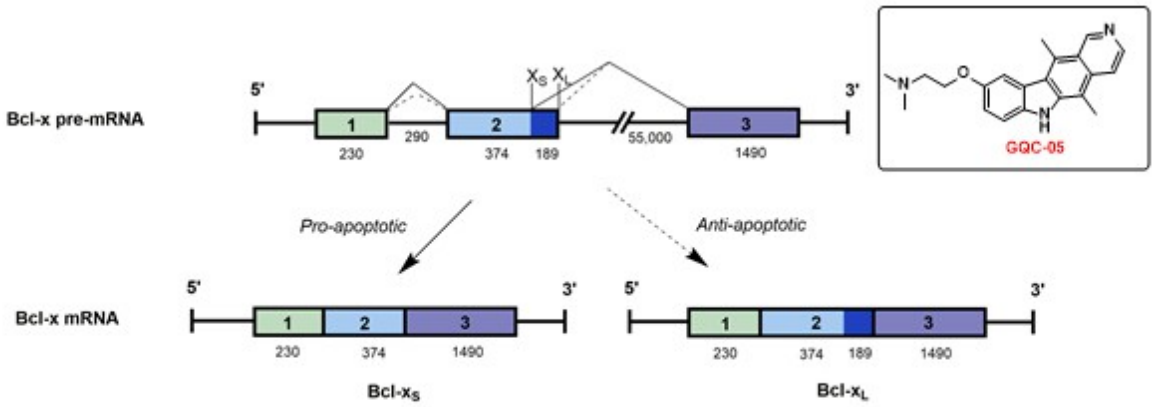

Figure 1: Alternative Splicing pathways pf Bcl-x towards the shorter isoform (Bcl-xS) and the longer isoform (Bcl-xL)

In this poster, we present a suite of small molecules that induce switching of the splicing pathway of Bcl-x in favour of the production of the pro-apoptotic Bcl-xS isoform.4 A focused structure-activity-relationship profile revealed key functional requirements for splice-switching activity of this suite of ellipticine compounds. Furthermore, we present a one-pot, modular route for the synthesis of ellipticine analogues that will allow us to access an extensive library of small molecules, that can be used to probe the mechanism of binding.

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TARGETING EZH2 FOR DRUG DISCOVERY: PROSPECTIVE EPIGENETIC INHIBITORS FOR CANCER THERAPY

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Epigenetic pathways are being recognized as determinants to cancer development and progression. Polycomb repressive complex 2 (PRC2) is an epigenetic regulator that catalyzes the trimethylation of lysine 27 in Histone 3 (H3K27me3), a process that facilitates chromatin compaction and gene silencing.[1] The overexpression of EZH2, the catalytic subunit of PRC2, is implicated in the development and progression of a variety of cancers with the worst prognosis. Thus, the therapeutic targeting of EZH2 emerged as a hot topic and the development of selective small-molecule EZH2 inhibitors is currently a promising research challenge for drug discovery.[2]

A combination of computational drug design techniques, synthetic methodologies and biological testing are being used to develop the new molecules. We performed a computer-aided drug design campaign to design new EZH2 inhibitors using LigandScout.[3] A panel of unique pharmacophore models were generated, validated and optimized. The prioritized models were used for two hit finding campaigns: Virtual Screening and *De Novo Design.* For the Virtual Screening approach, several databases (e.g., DrugBank, NCI, MuTaLig Chemotheca, and our in-house libraries) were computed and screened. Interesting virtual hit molecules with high inhibition potential were found and tested in order to determine their EZH2 profiles. Notably, we found several hits with inhibition rates comparable to the reference compounds (in clinical trials). In parallel, we started a *De Novo Design* campaign based on selected pharmacophore models and we found a new scaffold for EZH2 inhibitors. Those from *de novo design* were synthesized and tested. The potential toxicity issues were also assessed through metabolism studies. Finally, selectivity and binding mode of the most promising compounds are being elucidated. The best drug candidates are expected to proceed to *in vivo* testing.

Acknowledgements: This work was supported by grant PD/BD/128320/2017 from Fundação para a Ciência e a Tecnologia (FCT) and projects UIDB/00100/2020, UIDP/00100/202, PTDC/QUI-QAN/32242/2017, and SAICTPAC/0019/2015, funded by national funds through FCT and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. This communication is based upon work from COST Action CA15135, supported by COST.

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COARSE-GRAINED MOLECULAR DYNAMICS SIMULATIONS OF ANTIMICROBIAL PEPTIDES AGAINST MEMBRANE MODELS

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The increasing emergence of resistant bacteria is a great concern in terms of public health as available conventional antibiotics drugs are not able to kill them. One strategy proposed is the use of bacterial membranes as a therapeutic target so that their basic properties are perturbed, altering the membrane potential and inhibiting the control functions on the signalling, communication or production bioenergy processes. In this sense, antimicrobial peptides (AMPs) exhibit unique properties, which include broad-spectrum activity, rapid action and difficult development of resistance. They are part of the innate immune system in a large number of species, where they form the first line of defence against pathogenic invasion, still maintaining its effectiveness after being present in nature for thousands of years. Despite these advantages, AMPs have, in general, a small therapeutic window, and can hardly be used systemically because of their high toxicity. A detailed understanding of the molecular details of the membrane permeabilization process would allow the rational design of new molecules with the same mechanism of action, but with improved activity, selectivity, and bioavailability. Computational studies play an increasingly important role in understanding the structure and dynamics of biomolecular systems. For example, Molecular Dynamics simulations using coarse-grained (CGMD) resolution is able to systematically explore events that take place into ranges where direct comparison and experimental testing are starting to be feasible. In this study, we performed CG-MD simulations (5 μs) of series of AMPs in the presence of different membrane models containing different mixtures of1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) and 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), imitating bacterial, and 1-palmitoyl-2-oleoyl-snglycero-3-phosphocholine (POPC) representing mammalian membranes. The outcome of this study should provide the basis to design better AMPs that will disrupt the bacterial membrane and ultimately cause the death of their cells.

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SYNTHESIS OF GEMCITABINE 5'-PHOSPHORAMIDATE PRODRUGS IN THE NUCLEOSIDE DIMERS FORM

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Despite significant scientific advance, cancer is a leading cause of death worldwide. The increasing statistics are the result of several factors, including population growth and aging, as well as social and economic development. This increase is inevitable, which is why the development of research into cancer therapies is so necessary. Chemotherapy is one of the three basic therapeutic methods for this disease entity. Antimetabolites stand out among the cytostatic agent. They are a group of drugs that interfere with a specific metabolic pathway due to the displacement of the natural metabolite. Gemcitabine (2´,2´-difluoro-2´-deoxycytidine (dFdC)) is one of the interesting examples of this group of cytostatic agents. dFdC is the only cytidine analogue approved by the FDA that effectively affects solid tumours. Among others, it is used to treat metastatic pancreatic, ovarian or non-small cell lung cancer. Gemcitabine's basic mechanisms of resistance include intracellular transport, as well as biochemical processes in the intracellular space which are controlled by kinases [1]. Therefore, interesting structures are gemcitabine pronucleotides, which in the form of masked 5'-O-phosphates, allow to improve these pharmacodynamic and pharmacokinetic parameters. Our research is focused on the synthesis of dFdC prodrugs with a 5'-phosphoramidate structure. Here we report the synthesis of six gemcitabine prodrugs with the following carriers: n-propylamine and para-chlorophenyl. In addition, these derivatives were secured on the gemcitabine exoamino group with an additional nucleoside unit. We obtained hybrids in the Cu(I) catalysed Huisgen azide–alkyne 1,3-dipolar cycloaddition. The reaction substrate was 4-*N*-(propargyloxycarbonyl)-5'-phosphoramidate of gemcitabine and five 5'-azido gemcitabine derivatives with different protective groups and AZT, respectively [2]. We also present results of the initial evaluation of the cytotoxic activity of the obtained derivatives in five human cancer cell lines: cervical (HeLa), nasopharyngeal (KB), liver (HepG2), lungs (A-549), glioblastoma (U87), and also normal human dermal fibroblast cell line (HDF).

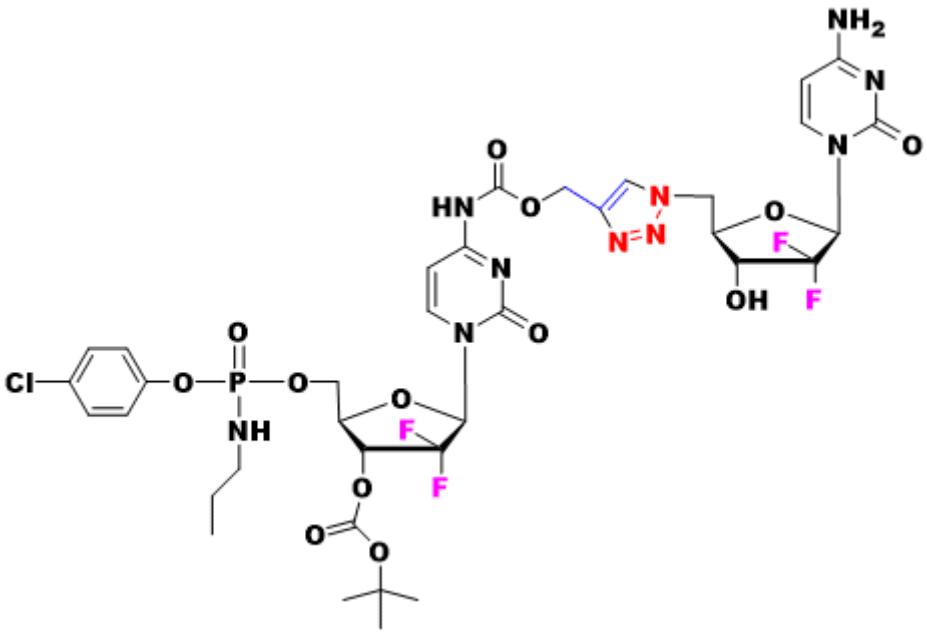

Figure 1. An example of gemcitabine prodrug.

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DESIGN AND IDENTIFICATION OF A GPR40 FULL AGONIST (SCO-267) POSSESSING A 2-CRRBAMOYLPHENYL PIPERIDINE MOIETY FOR THE TREATMENT OF TYPE 2 DIABETES MELLITUS

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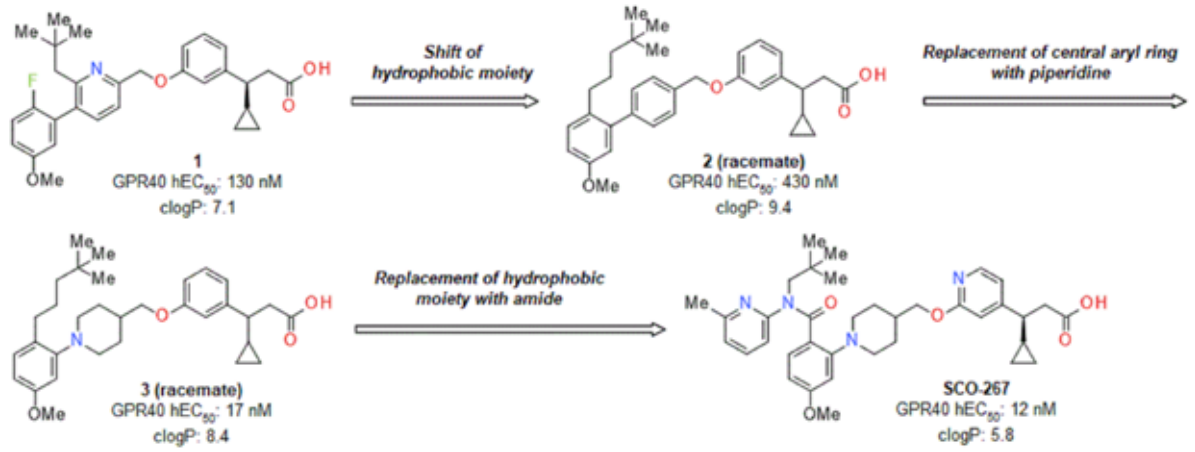
GPR40/FFAR1 is a G-protein-coupled receptor expressed in pancreatic β-cells and enteroendocrine cells. Full activation of GPR40 stimulates secretions of insulin and incretin, both of which are the pivotal regulators of glycemic control. Therefore, a small molecule full agonist of GPR40 is an attractive target for the treatment of type 2 diabetes mellitus. Using the reported biaryl derivative **1**, we shifted the hydrophobic moiety onto the terminal aryl ring and replaced the central aryl ring with piperidine, generating 2-(4,4-dimethylpentyl)phenyl piperidine **3**, which had improved potency for GPR40 and high lipophilicity. We replaced the hydrophobic moiety with *N*-alkyl-*N*-aryl benzamides to lower the lipophilicity and restrict the *N*-alkyl moiety to the presumed lipophilic pocket using the intramolecular π-π stacking of *cis*-preferential *N*-alkyl-*N*-aryl benzamide. Among these, orally available (3*S*)-3-cyclopropyl-3-(2-((1-(2-((2,2-dimethylpropyl)(6-methylpyridin-2-yl)carbamoyl)-5-methoxyphenyl)piperidin-4-yl)methoxy)pyridin-4-yl)propanoic acid **(SCO-267)** effectively stimulated insulin secretion and GLP-1 release and ameliorated glucose tolerance in diabetic rats via GPR40 full agonism. A phase 1 study of **SCO-267**, a first-in-class oral GPR40 full agonist, has been initiated in healthy adults and people with impaired glucose tolerance. In this presentation, we will describe the design, synthesis, and pharmacological effect of **SCO-267** as a potent and orally bioavailable GPR40 full agonist.



DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF SMALL MOLECULES TARGETING THE PI3K/AKT/mTOR PATHWAY FOR GLIOBLASTOMA TREATMENT

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Glioblastoma multiforme (GBM) is the most common malignant brain tumor, and it is associated with poor prognosis with a median overall patient survival of 12 – 16 months from diagnosis and a survival rate of 3 – 5% for a period of 3 years or more.[1] Despite the tremendous progresses carried out on the development of effective drugs for the treatment of certain cancers such as leukemias or breast cancer, GBM remains an unmet clinical need with only one approved drug (temozolomide) in the market.

Committed to the search of new alternative treatments, our group employed a phenotypic screening campaign to identify active molecules against GBM. The analysis of an in-house 100-compound library highlighted a series of 4-amino-pyrazolo[3,4-*d*]pyrimidine derivatives with potent antiproliferative activity against U87 and T98 glioma cell lines.[2] The most active hits of this set belonged to a group of compounds known to selectively target the PI3K/AKT/mTOR signaling pathway,[3] which has been reported to be affected in GMB. We sought to employ these 4-amino-pyrazolo[3,4-*d*]pyrimidine derivatives and other structural related analogs to optimize the interaction of this core with the PI3K/AKT/mTOR pathway and generate more effective drugs for the treatment of GBM.

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IDENTIFICATION AND EVALUATION OF NOVEL INHIBITORS TO TARGET PLASMODIUM FALCIPARUM

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Owing to the increased resistance to commonly used anti-malarial drugs as well as the newly found resistance to Artemisinin-based therapy, it has become of utmost importance to develop new malaria treatments that are both structurally and mechanistically different to previous medicines.[1] This project makes use of primarily a computationally-based approach to investigate new potential druggable targets within the malaria parasite *Plasmodium falciparum*. Of particular interest is dihydroorotate dehydrogenase (*Pf*DHODH), an enzyme which plays a key role in the parasite's *de novo* pyrimidine biosynthesis pathway.[2] The unique structure of this enzyme compared to its mammalian counterpart and the central role it plays in parasite survival, make it a viable target of future antimalarials. In an effort to identify potential *Pf*DHODH inhibitors, a two-dimensional map of antimalarial chemical space was generated using principle component analysis and a total of 207 molecular descriptors.[3] Percentage enrichment maps plotted using compounds of known *Pf*DHODH inhibitory activity where then used to qualitatively predict the inhibitory activity of numerous other compounds (obtained from ChemDiv libraries) that were also plotted onto the map.[4] The model predictions were validated by (1) using an extensive receptor-ligand docking study in which the *Pf*DHODH protein crystal structure was probed for potential inhibitor binding sites and compounds were docked using a variety of protocols and (2) making a selection of fifteen compounds with varying predicted *Pf*DHODH activities and getting them tested against both the *Pf*DHODH enzyme and parasite. Of the fifteen compounds, two showed *in vitro* enzyme inhibition, with one also showing *in vivo* parasite inhibition. A significant hit rate of 33.33% was achieved when following this protocol if a docking score cutoff of -10.00 kcal/mol was applied. Thus far, the combination of principle component analysis with molecular modelling of receptor-ligand interactions has shown promise as a means to identify new inhibitors of *Pf*DHODH.

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EVALUATION OF PLANT PHENOLS AS TLR4 SIGNAL TRANSDUCTION PATHWAY MODULATORS

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Toll-like receptors (TLRs) play a key role in the innate immunity. Activation of TLRs induces production of multiple proinflammatory cytokines. The hyperactivation of TLR-dependent cellular responses can cause development or progression of inflammation-associated pathological states. Therefore, suppression of the TLR-dependent cellular response could reduce excessive inflammation. Some natural products, including flavonoids, are known to modulate the activity of TLR-dependent signal transduction pathways. Secondary plant metabolites could be a good source for a search for novel TLR-induced inflammation modulators. This process can be simplified and accelerated by virtual screening and molecular modelling techniques. When looking for regulators for TLR4-dependent signal pathways, we evaluated possible molecular interactions of plant phenols with proteins involved in TLR4-dependent signal transduction using molecular docking.

We selected the set of plant phenols (200 compounds) using Dr. Duke's Phytochemical and Ethnobotanical Databases, PhytoHub and Phenol-Explorer. Conformational isomers of selected compounds were prepared using Python scripts and RDKit. Human proteins involved in TLR-dependent signal transduction were used as targets. Experiment-based 3D models of targets were taken from Protein Data Bank. Molecular docking was performed using Autodock Vina software. Values of ΔG for ligand-protein complex formation were used as scores and pKd values were calculated from ΔG. Ligand-protein complexes had been interpreted as tight-binding if pKd values were equal to or higher than 6.

Molecular docking produced a relatively small number of tight-binding phenol-protein complexes. TLR4 and five downstream proteins from TLR4 signal cascade were identified as high-affinity targets for plant phenols. Five phenols, including hesperidin, were predicted as high-affinity ligands for the extracellular domain of TLR4. In contrast, intracellular (TIR) domain of TLR4 formed no tight-binding complexes with any studied phenols. IRAK4, the first kinase of MyD88-dependent cascade, was predicted to have a high affinity for 10 plant phenols, including epigallocatechin 3-O-gallate (EGCG), hesperidin and inulin. Only 4 phenols were predicted as high-affinity ligands of TAK1 which is a kinase involved in NF-kB activation. Mitogen-activated protein kinase (MAPK) can be activated by TAK1 and is implicated in AP-1 transcription factor activation. Calculations showed that 5 phenols, including EGCG, hesperidin, quercetin and inulin, can form tight-binding complexes with MAPK. 8 phenols, including EGCG, hesperidin, quercetin and inulin, were predicted to tightly bind to NF-kB transcription factor. Kinase IKK, which activates NF-kB via phosphorylation of IkB inhibitor protein, predicted to have a high affinity for 10 plant phenols, including EGCG, quercetin and inulin.

In summary, 18 plant phenols were predicted as high-affinity ligands for components of TLR4-dependent signal transduction pathway. 10 of them could interact with 1 or 2 target proteins in this cascade. The maximal number of possible targets (4 out of 6 proteins) was identified for 5 phenol compounds such as EGCG, inulin, hesperidin, casurinin and tellimagrandin II. EGCG and inulin had the highest average affinity for their protein targets among tested plant phenols.

For experimental validation of molecular docking modelling, we chose to test EGCG and extract of sage (Salvia officinalis L.) with a high content of various flavonoids and other phenols. The effect of these compounds on TLR4-dependent cellular response was measured through changes in phagocytic activity of intact (control) and lipopolysaccharide (LPS)-stimulated (TLR4 activated) human oral neutrophils. Based on the results of this assay the percentage of activated neutrophils (PAN) and the phagocytic index (PhI) were calculated. 1 μM EGCG significantly suppressed LPS-stimulated (PAN -21.2% and PhI -11.7%) but not the basal phagocytic activity of neutrophils. In contrast, the extract of sage containing a wide spectrum of phenols (final concentration: 3 μM flavonoids and 8.6 μM phenols) decreased both LPS-stimulated (PAN -51.4% and PhI -13.6%) and basal (PAN -37.0% and PhI -5.9%) phagocytic activity.

Hence, molecular docking technique can be successfully used for primary virtual screening of possible modulators of TLR4-dependent signal pathways and the results could be validated using a simple and inexpensive test based on phagocytic activity of LPS-stimulated human oral neutrophils.

TARGETED THERAPY FOR NEUROLOGICAL DISORDERS: A NOVEL, ORALLY AVAILABLE, AND BRAIN-PENETRANT MTOR INHIBITOR (PQR626)

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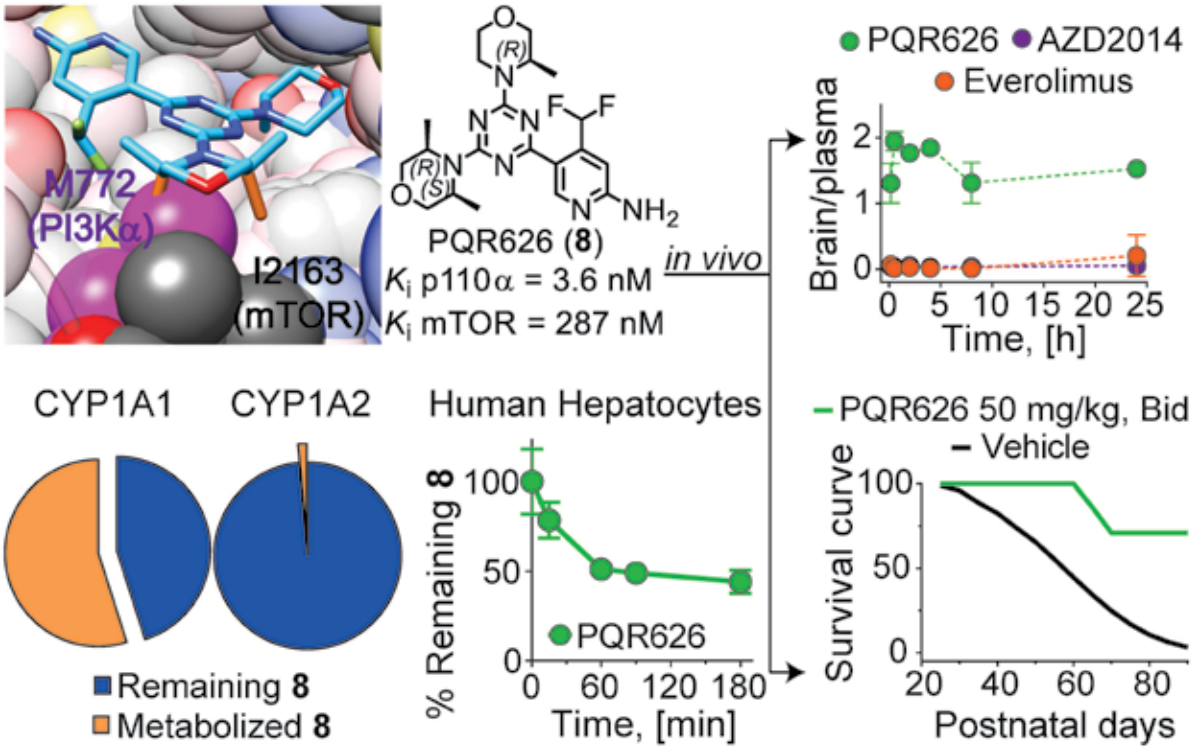
Mechanistic target of rapamycin (mTOR) regulates cell proliferation, growth and survival, and is overactivated in cancer and neurological disorders [1]. Rapamycin derivatives (rapalogs) and mTOR kinase inhibitors (TORKi) have recently been applied to alleviate epileptic seizures in Tuberous Sclerosis Complex (TSC) [2].

Herein, we describe a pharmacophore exploration to identify a highly potent, selective, brain penetrant TORKi with optimized metabolic stability. An extensive investigation of the morpholine ring engaging the mTOR solvent exposed region led to the discovery of PQR626. PQR626 displayed excellent brain penetration as compared to everolimus and AZD2014 in rats and mice (brain:plasma ratio of ~ 1.8:1 for PQR626; ~ 1:61 for everolimus; ~ 1:25 for AZD2014). PQR626 was well tolerated in mice (MTD: 100-150 mg/kg). A dose-range finding efficacy study in mice with a conditionally inactivated *Tsc1* gene in glia (*Tsc1*^{GFAP}CKO mice) showed a significant reduction of loss of *Tsc1*-induced mortality at 50 mg/kg PQR626 (p.o. BID, twice a day).

PQR626 overcomes the metabolic liabilities of PQR620 [3], the a first-in-class brain penetrant *TORKi* showing efficacy in a TSC mouse model. The improved stability in human hepatocytes, together with the excellent brain penetration, safety profile and efficacy in *Tsc1*^{GFAP}CKO mice qualify PQR626 as a potential therapeutic candidate for the treatment of epilepsy and neurological disorders.



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A CASE STUDY ON ADENOSINE RECEPTORS LIGANDS: TAXICAB GEOMETRY, IN SILICO/IN VITRO QUANTIFICATION SYSTEM - FROM EVALUATION TO VALIDATION

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As an outcome of previous studies [1] multiple adenosine A1 receptor ($A_{1A}R$) homology models had been obtained and a library of lead-like compounds had been docked, that resulted with an identification of number of potent and one selective ligand toward the intended target. However, in *in vitro* experimental verification studies, many ligands also bound to the $A_{2A}AR$ and the A_3AR subtypes. In this work we asked the question whether a classification of the ligands according to their selectivity was possible based on docking scores. Therefore, an A_3 AR homology model was built and all previously found ligands to all three receptor subtypes were docked. As a metric, we employed an *in vitro/in silico* selectivity ranking system based on taxicab geometry and obtained a classification model with reasonable separation. In order to test the usability and versatility of the described method, a library of 88 selective ligands previously described by Katritch et al. [2] was used with similarly good performance.

Acknowledgements

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THE HEME DETOXIFICATION PATHWAY: DEVELOPMENT OF A MODEL SYSTEM FOR THE IDENTIFICATION AND VALIDATION OF NEW INHIBITORS

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The heme detoxification pathway is well-established as an effective antimalarial drug target. Recent high throughput β-hematin inhibition screening of large data has opened doors to new methods of drug discovery. In one study, 13214 compounds from the Tres Cantos Antimalarial dataset (TCAMS), were screened for β-hematin inhibition.

Aim

The development and validation of a principle components analysis (PCA)-based 2-D model of antimalarial chemical space based on high-throughput β-hematin inhibition screening data for the identification of new β-hematin inhibitors.

PaDEL was used to calculate descriptors for the 13214 compounds; thereafter Matlab was used for carrying out PCA and plotting the data as a 2-D map. Three areas on the map were selected, of which 36, 29 and 0.1% of compounds in the respective areas showed activity. New compounds were plotted onto the map and from these areas, 90 selected to be tested for β-hematin inhibition using the NP-40 method.

From the three respective areas 37.5, 30 and 3.3% of compounds had IC_{50} values less than 150 μM showing agreement with the predictions from the PCA model.

This illustrates the potential of using such a PCA model in narrowing down the search for new inhibitors and potential antimalarials.

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DEVELOPMENT OF FLEXIBLE ARYLSULFONAMIDES AS POTENTIAL MULTITARGET ANTICANCER AGENTS

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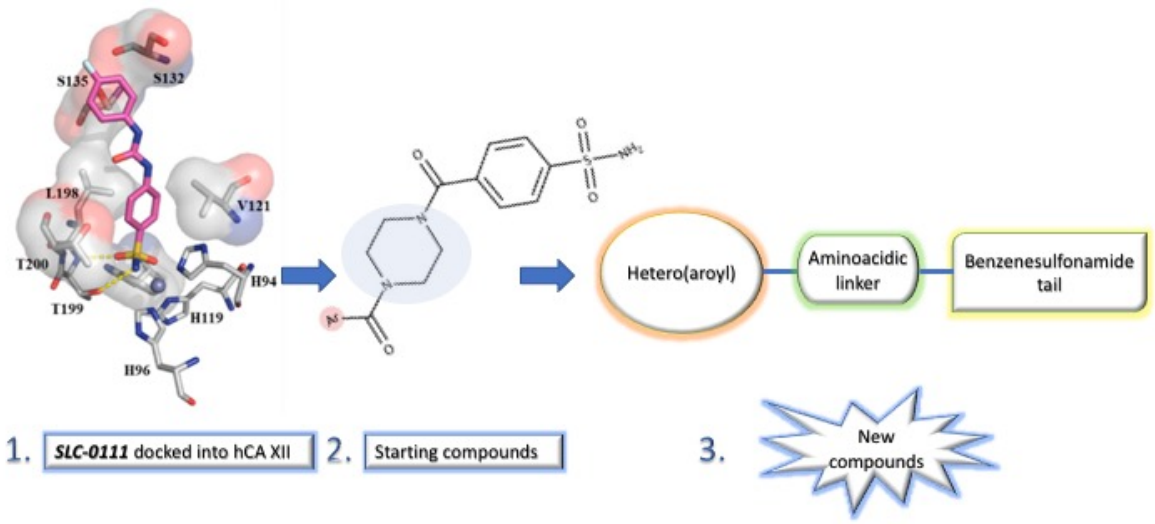
Several compounds containing arylsulfonamide moiety have been reported in literature as potential anticancer agents and the most advanced molecule is the 4-ureidobenzenesulfonamide derivative SLC-011 (WBI-5111), which recently entered in clinical trials for the treatment of hypoxic tumors in the metastatic pancreatic ductal cancer.[1] The anticancer effects of SLC-0111 are related to the inhibition of the tumor-associated human carbonic anhydrases (hCAs) isoforms IX and XII[2] which catalyze the reversible hydration of carbon dioxide to bicarbonate and proton thus controlling the pH of tumor microenvironment (TME), which differs from normal tissue. It seems that hCA IX and hCA XII contribute thus maintaining a moderately alkaline cell pH and this acidification promotes local invasion and metastasis. Given the role of hCA IX and XII in cancer therapies, this class of CA inhibitors might offer therapeutic opportunities to obtain multitarget agents and overcome the drug resistance, which might occur in cancer cell. Looking for new hCA IX/XII inhibitors,we identified several 4-(4-aroylpiperazine-1-carbonyl)benzenesulfonamides, that exhibited K_i values in the low nanomolar range.[3] Encouraged by these promising results we designed a series of heteroaroylanalogs focused on modifying the 4-carbonylpiperazine core with a more flexible amino acetamide linker to evaluate interactions in the middle/top area of the hCA active pocket formed by lipophilic aminoacid side chains.



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REDOX PROPERTIES, ROS GENERATION AND ANTIPROLIFERATIVE ACTIVITY OF FERROCENE-SUBSTITUTED NUCLEOBASES

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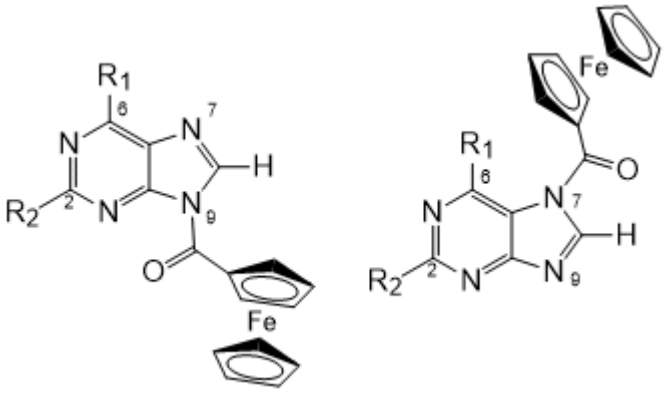
Ferrocenyl compounds are shown to exhibit diverse biological activity through a redox mechanism by generating reactive oxygen species (ROS).[1] Since ferrocene-substituted nucleobases combine biogenic and redox-active parts, they have attracted the substantial attention and been investigated in medicinal chemistry to develop new candidates for therapeutic use.[2]

To examine the biological activity of ferrocene-substituted nucleobases, we have prepared a series of ferrocene-substituted purine-nucleobase derivatives [3], measured their redox potential by the cyclic voltammetry and carried out an acellular ROS generation tests followed by in vitro cytotoxic studies on L929 and selected cancer cell lines. As expected, all measured compounds showed a reversible one-electron oxidation in the range of 300-450 mV with significant difference of approximately 100 mV between the N7 and N9 regioisomers allowing to easily distinguish them. The tendency of ferrocene-nucleobase conjugates to produce reactive oxygen species (ROS) was examined by DCFH assay in DMSO. The results showed that the nucleobases coupled with ferrocene generate ROS, while neither ferrocene itself nor the nucleobases were active. Finally, antiproliferative activity of compounds was examined using MTS viability assay of L929 cells and cancer cells HepG2 and Panc-1 to determine cytotoxic effect based on the IC_{50} values. Both the ability to produce acellular ROS as well as the IC_{50} are affected by the substituents on the purine ring.



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NANOCYCLIX® ALK2 INHIBITORS TO OVERCOME CANCER-INDUCED ANEMIA

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Introduction: Anemia is a condition characterized by low Hemoglobin levels. Anemia is observed during tumor development through the release of inflammatory cytokines, which will affect iron availability, a key component of red blood cells. In recent years, consequences of anemia of cancer on patients is getting growing recognition from a quality of life perspective, as well as its impact on the treatment scheme and hospitalization duration. Cancer-induced anemia has been identified in 30% of treatment-naive cancers (ECAS study, 2011 – WHO methodology). The proportion of anaemic patients reaches 70% following treatments, such as chemotherapies. Current approaches consider erythropoietin derivatives, formulated iron or transfusions, which does not address underlying chronic inflammation. Moreover, their use is limited as a consequence of toxicities (EPO, formulated iron) or availability (transfusion).

Increased expression of Hepcidin, a peptidic hormone regulating the storage of bioavailable iron, has been demonstrated as downstream effector of key inflammatory cytokines activation of ALK2/Smad axis. ALK2 (Activin-like receptor kinase 2) plays a critical role in the Smad signaling pathway and the production haemoglobin, through the regulation Hepcidin production in the liver. As a consequence, the selective inhibition of ALK2 as emerged as a valuable target for the treatment of Hepcidin-driven anemia of cancer to provide an alternative to current treatments.

Results: OD52, a potent and selective macrocyclic ALK2 inhibitor identified from Nanocyclix® technology platform, was used as *in vitro* pharmacological tool to investigate the impact of ALK2 inhibition on Hepcidin expression level in HepG2 cells, as well as in mice hepatocytes. BMP6-induced expression of Hepcidin mRNA was completely inhibited by LDN-193189 to low detectable levels, whereas OD52 normalized its expression. Further validation was performed in mice hepatocytes, where OD52 decreased Hepcidin expression to a lower extend as compared with LDN-193189. These experiments confirmed the importance of selectivity within BMP-kinase receptor family, where ALK3 component represents an undesired off-target.

In vivo evaluation of the compound in the acute model of turpentine-induced anemia demonstrated a normalization of haemoglobin level. In contrast, LDN-193189 increased haemoglobin above vehicle group to a level mimicking hemochromatosis, likely due to its ALK3 component.

Through further medicinal chemistry optimization, OD66 was identified as suitable *in vivo* pharmacological tool and was evaluated in a cancer-induced anemia mouse model. The compound demonstrated a normalization of haemoglobin levels to comparable levels with the tumor-free group without displaying toxicity.

Conclusion: The identification of potent and selective Nanocyclix® ALK2 inhibitors and their evaluation in relevant *in vitro* and *in vivo* models demonstrated a normalization of haemoglobin levels through the modulation of Hepcidin expression. This collaborative work warrants further optimization towards the identification of a preclinical candidate.

STRUCTURAL OPTIMIZATION AND IN VITRO EVALUATION OF NEW 1-(4-FLUOROBENZYL)PIPERAZINE DERIVATIVES AS INHIBITORS OF TYROSINASE FROM AGARICUS BISPORUS

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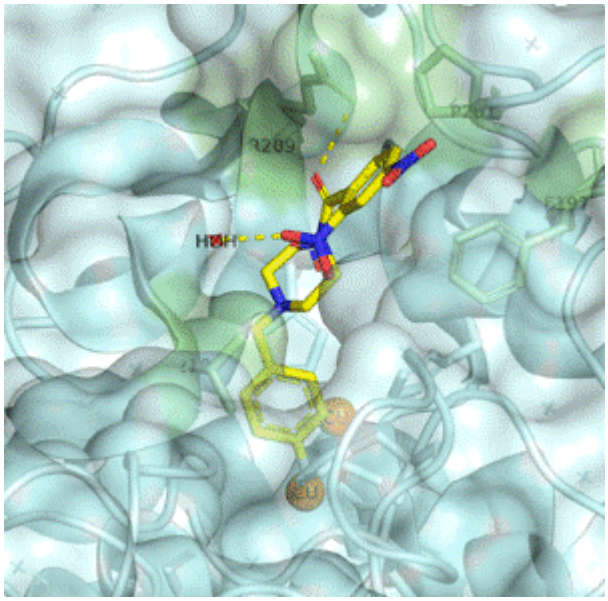
Tyrosinase (TYR, EC 1.14.18.1) is a metallo-enzyme widely distributed among various organisms (bacteria, fungi, plants, and mammals). The tyrosinase family includes different TYR isoforms sharing the active central domain that is composed of two copper ions coordinated with six histidine residues. The physiological role of TYRs is the conversion of L-tyrosine to dopaquinone as the limiting reaction for the melanin biosynthesis in melanogenesis.

Therefore, a lot of natural and synthetic tyrosinase inhibitors (TYRIs) have been developed in order to identify therapeutic strategies against skin hyper-pigmentation and melanoma in humans.[1] In the past years, we have developed a large series of (hetero)arylcarbonyl compounds [2-4] bearing the 1-(4-fluorobenzyl)piperazine fragment as structural motif for inhibitory properties toward tyrosinase from *Agaricus bisporus* (a.k.a. mTYR) which is readily available at low cost and represents the best surrogate of human tyrosinase (hTYR) in biochemical assay.



The (2,4-dinitrophenyl)(4-(4-fluorobenzyl)piperazin-1-yl)methanone (**I**) proved to be a potent inhibitor toward mTYR (IC_{50} = 0.96 μM) and showed a competitive mechanism of action. Thanks to theoretical and crystallographic studies on TYR from *Bacillus megaterium*, we have demonstrated that the 4-fluorobenzylpiperazine fragment plays a key role for the binding into the tyrosinase catalytic site close to copper centers (figure). Moreover, it has been shown that the remaining structural portion of the best active inhibitors establishes favourable contacts in the enzymatic cavity.

Herein we report the synthesis of novel 1-(4-fluorobenzyl)piperazine-based compounds as structural homologues of prototype **I**; the new compounds were designed by inserting an additional methylene group into the aroyl fragment and selecting newer molecules bearing -NO_2 and/or -CF_3 substituents on aromatic ring. After the biochemical screening toward mTYR we identified a further series 4-fluorobenzyl-based compounds that proved to be effective mTYR inhibitors at sub-micromolar concentration.

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SUSTAINABLE SYNTHESIS OF NEW CHOLINE KINASE INHIBITORS

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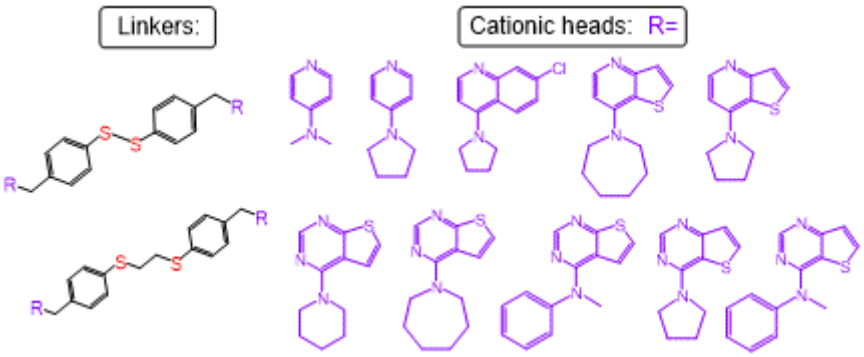
Keywords: *Cancer, choline kinase, green chemistry.*

The need for new safer and greener chemical pathways is a strong tendency in nowadays drug discovery industry. [1] Not only environmental aspects but also economic ones, are taken in account while designing a synthetic pathway.

Lately, we have been interested in the synthesis of bioisosteric inhibitors of the enzyme Choline Kinase. This cytosolic enzyme is established as a new therapeutic target because of its overexpression in ras-transformed cells. Its role is the catalytic transformation of choline into phosphatidylcholine by using Mg^{2+} and ATP in the first step of the Kennedy pathway. As a result, the increase of phosphatidylcholine, which is one of the principal components of the plasma membrane of eukaryotic cells, allows cell proliferation and is associated with tumorigenesis.Studies using RMN analysis, have recognized this effect in a lot of types of cancer as bladder, breast, colon, and prostate. [2] So the synthesis of ChoK inhibitors has opened a promising window to common therapies for cancer. Our group has focused on the synthesis of new inhibitors that are characterized for the bioisosteric exchange [3, 4] of some carbons to sulphur atoms, giving room to better interaction with the amino acidic residues of the enzyme pocket and conserving the antiproliferative activity of the previous ones. The synthesis of such molecules has been performed in a sustainable and efficient way, paying special attention to the use of reaction conditions able to generate the minimal amount of waste. Good yields in each step and no purification of intermediates make of this synthesis a good approach towards sustainability.



Biological assays are currently being performed to confirm our hypothesis.

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NOVEL DUAL D3R/GSK-3β MODULATORS: AN INNOVATIVE MULTITARGET STRATEGY FOR BIPOLAR DISORDER

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Among the psychiatric diseases, bipolar disorder (BD) is the sixth leading cause of disability with a prevalence up to 4 % worldwide. BD is a severe neuropsychiatric condition which alternates episodes of mania with symptoms of depression. Although the neurobiological pathways are not completely clarified, the dopamine (DA) hypothesis has been recognized the leading theory able to explain the pathophysiology of the malady. In detail, faulty homeostatic regulation of dopaminergic circuits is likely to play a role in cyclical and marked changes of DA neurotransmission, which are at the basis of the bipolar nature of the disorder[1]. Modulation of D2 and D3 receptors (D2/3R) through partial agonists represents the first-line therapeutic strategy for psychiatric diseases[2]. Moreover, a deregulation of the enzyme glycogen synthase kinase-3β (GSK-3β) has been reported as peculiar feature of BD[3,4]. In this scenario, the concomitant modulation of D3R and GSK-3β, by employing multifunctional or multitarget compounds, could offer promises to achieve an effective cure of this illness. In the light of these findings, we rationally envisaged the pharmacophoric model at the basis of the design of several D3R partial agonists, suitable to be exploited for the dual D3R/GSK-3β ligand design. Thus, synthetic efforts were addressed to develop a first set of hybrid molecules able to concurrently modulate the selected targets. For a chemical structure point of view, we employed different spacers to combine a substituted aryl-piperazine moiety, reported in previously discovered D3R modulators[5], with a pyrazole-based fragment, already identified in GSK-3β inhibitors[6]. A fluorescent and a cellular functional assays were carried out to assess the activity of all synthetized compounds against GSK-3β and on D3R, respectively. Most of the derivatives proved to effectively modulate both GSK-3β and D3R with potencies in the low-μM and low-nM range, respectively. The consistent biological data obtained allowed us to identify some lead candidates worth to be further modified with the aim to optimize their biological profile and to perform a structure-activity relationship (SAR) study[7].

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DISCOVERY OF REVERSIBLE MAO-B INHIBITORS DERIVED FROM DIARYL 2-BUTENAMIDES

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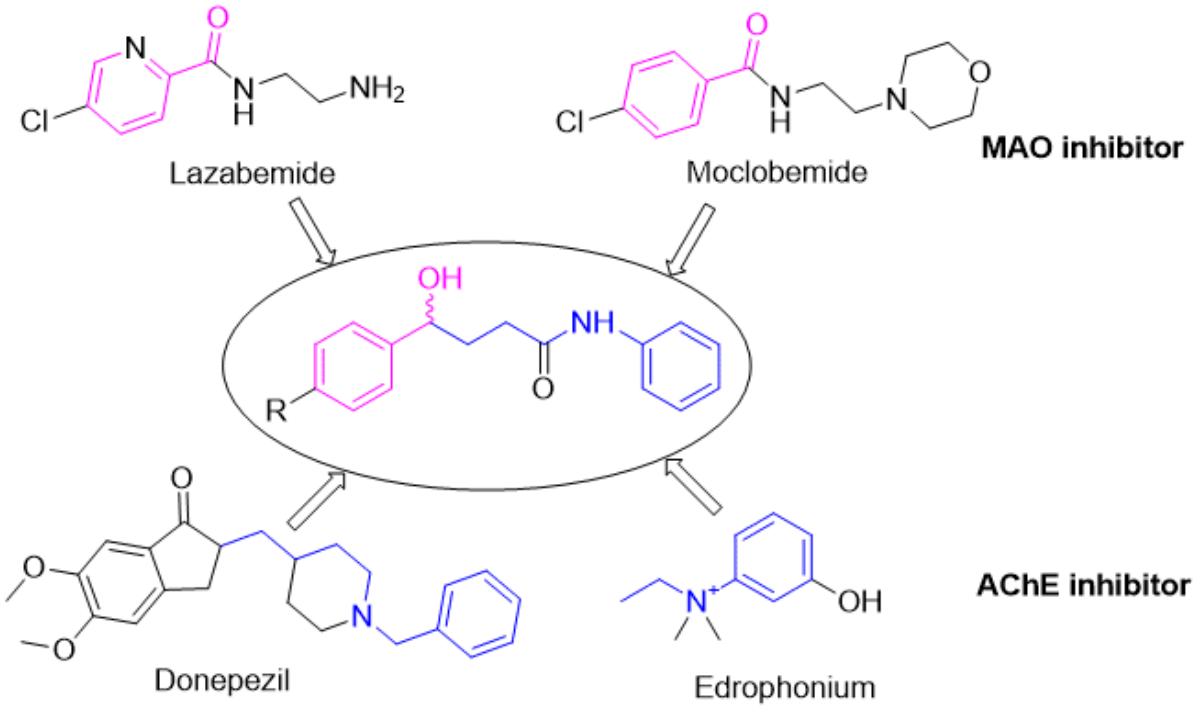
Small molecules as monoamine oxidase-B inhibitor, along with cholinergic inhibition, act as essential ligands for the treatment of various neurological disorders, *e.g.,* Alzheimer's dementia, Parkinson's disease, *etc*. Fragment-based drug design provides the diaryl-4-hydroxy-2-butenamide derivatives, afforded reversible MAO-B inhibitors with cholinesterase inhibition activity. Compounds **7k**, **7s**, **7u**, & **7v** showed reversible potent MAO-B inhibition, and compounds **7k**, **7u**, and **7v** also provided AChE inhibition (IC_{50}) within nanomolar range. The substitution at *para*-position of a 4-phenyl moiety of aryl-4-phenyl but-2-enamides with hydroxyl group showed better reversible MAO-B inhibition activity than others. Further, the substitution at *para*-position of 4-phenyl moiety with OH group showed better inhibition activity than other groups (H, CH_3, Cl, and OMe). Molecular dynamics studies also help to explain the drug-enzyme interactions. The synthesized compounds showed remarkable BBB permeability, neuroprotectivity against L-glutamate induced excitotoxicity and no toxicity on human neuroblastoma SH-SY5Y cell line.



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PREDICTION AND VALIDATION OF A DRUGGABLE SITE ON VIRULENCE FACTOR OF DRUG RESISTANT BURKHOLDERIA CENOCEPACIA

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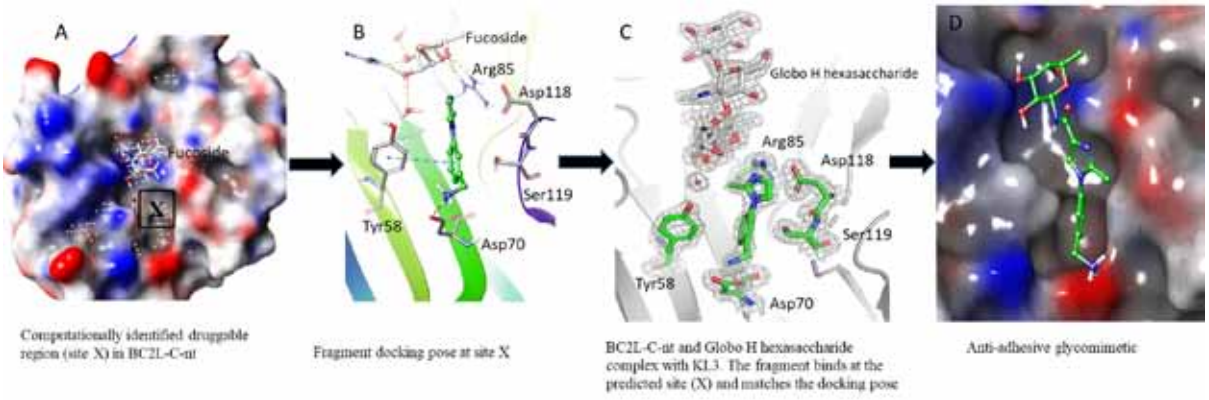
The emergence and spread of multidrug-resistant (MDR) bacteria have challenged the existing treatment regimen which has enormous implications for worldwide healthcare delivery and community health.[1] *Burkholderia cenocepacia* is a gram-negative bacterium responsible for the deadly lung infection in patients with immunocompromised conditions like chronic granulomatous diseases and cystic fibrosis.[2] The strains of *B. cenocepacia* show extreme resistance to almost all clinically useful antibiotics,[3] being thus responsible for significant morbidity and mortality. *B. cenocepacia* produces a lectin called BC2L-C which act as a virulence factor responsible for the host cell infection.[4] BC2L-C binds to the carbohydrates present on the epithelial cells of the host and reported to be involved in the bacterial adhesion and subsequent infection process. The N-terminal domain (BC2L-C-nt) of the lectin has been characterized as a novel fucose-binding domain with a TNF-α-like architecture while the C-terminal domain specifically binds to the mannose.[5-6] Thus, BC2L-C represents a novel type of super lectin with dual specificity for fucose and mannose in the N- and C-terminal domains, respectively. Therefore, BC2L-C-nt is an interesting target for designing the drug molecules for the anti-adhesive therapy to prevent lectin mediated bacterial adhesion to the host epithelium. A virtual screening campaign and a series of biophysical assays identified the molecular fragments binding at the proposed druggable region labelled as X (Fig.)near the fucose binding site.



The X-ray crystallographic screening further validated the binding affinity of the identified hits at the site X. The identified molecular fragments have been linked synthetically to the fucose to convert into high-affinity glycomimetics. The structure-based strategies further provide opportunities to elaborate the studies on the lead molecule to design a potent glycomimetic.

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SYNTHESIS OF ROM-ANALOGUES FOR BIOLOGICAL STUDIES

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The natural compound rhodomyrtone (Rom), which was isolated from *Rhodomyrtus tomentosa* leaves in 2002[1], shows a high antimicrobial activity against Gram-positive bacteria among them multi-resistant *Staphylococcus aureus* and different types of *Streptococcus*[2]. Investigations on a Rom-resistant strain of *Staphylococcus aureus* assume, that a Rom resistance is mediated by the overexpression of the FarE-Protein[3], an efflux pump for linoleic and arachidonic acids[4]. To explore the substrate specificity for FarE, we want to synthesize Rom-analogues, which will be tested for antimicrobial activity, cytotoxicity and solubility and also on the resistant Rom^R mutant.

Rhodomyrtone (Rom) Rom-analogues

After investigating the structure-activity relationship, we want to link the Rom-analogues with sugars, dipeptides or by adenylylation.

Since Rom penetrates the cell wall to attack the bacterial membrane, we want to synthesize analogues, which can be detected in the migration process by time-lapse fluorescence microscopic analysis.

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ISOLATION AND PHARMACOLOGICAL EVALUATION OF Δ9-TETRAHYDROCANNABIPHOROL, THE NEW HEPTYL HOMOLOGUE OF TETRAHYDROCANNABINOL: NEW INSIGHTS ON CANNABIS RESEARCH

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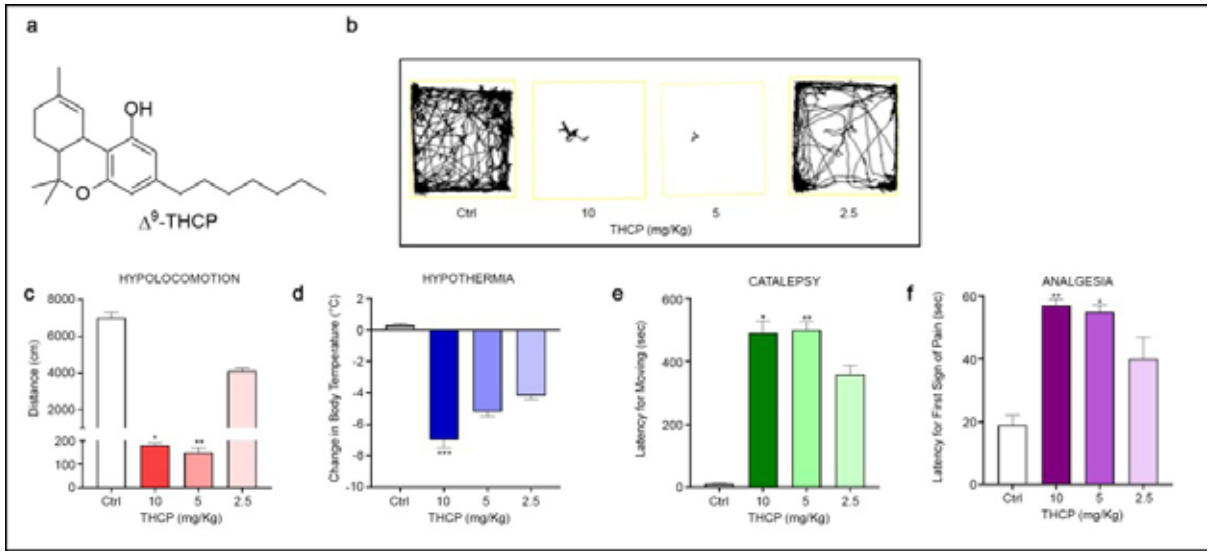
Cannabis sativa L. produces characteristic terpenophenolic compounds called phytocannabinoids, among which the most studied is Δ^9-tetrahydrocannabinol (Δ^9-THC). The wide structural variety of such compounds derives from the combination of a terpene and a resorcinyl moiety, the latter bearing an alkyl side chain.[1] It has been reported that the length of this carbon chain influences the biological activity of tetrahyddrocannabinol.[2] In particular, synthetic analogues of Δ^9-THC with a longer side chain have shown cannabimimetic properties far higher than Δ^9-THC itself.[3] To date, no phytocannabinoid with a linear side chain longer than five carbon units has been reported. In this work, a new phytocannabinoid with the same structure of Δ^9-THC but with a seven-term alkyl side chain was identified in a medicinal cannabis variety. Isolation, full characterization and stereochemical configuration assignment of the natural compound were carried out. This new phytocannabinoid has been called (-)-*trans*-Δ^9-tetrahydrocannaphorol (Δ^9-THCP) (Fig. 1a). Similarly, the heptyl homologue of the well known phytocannabinoid cannabidiol (CBD), named cannabidiphorol (CBDP), was unambiguously identified. The binding activity of Δ^9-THCP against human CB_1 receptor *in vitro* resulted over 30-fold higher than that of Δ^9-THC (K_i=1.2 nM *vs* K_i=40 nM respectively). *In vivo* tests showed that Δ^9-THCP reduced locomotion (Fig. 1b-c), decreased rectal temperature (Fig. 1d) and induced catalepsy (Fig. 1e) and analgesia (Fig. 1f) suggesting a THC-like cannabimimetic activity.



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IDENTIFICATION OF DIHYDROQUINOLONE PYRAZOLINE BASED-COMPOUND AS RAD51-BRCA2 INTERACTION DISRUPTOR TO INDUCE SYNTHETIC LETHALITY IN PANCREATIC CANCER

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Synthetic lethality is an innovative framework for the discovery of new anticancer drugs. Cancer cells are strictly dependent on efficient DNA repair pathways due to their pervasive genomic instability. [1] Targeting different DNA repair pathways provides the opportunity to apply the synthetic lethality as a novel anticancer therapeutic strategy. One straightforward example of this is the use of the PARP inhibitor (PARPi) Olaparib, to treat BRCA2-defective cancers. [1] In this context, we proposed to trigger a fully small-molecule-induced synthetic lethality, combining a RAD51-BRCA2 protein-protein interaction (PPI) disruptor with Olaparib to target pancreatic cancer, one of the major unmet oncological needs. RAD51-BRCA2 PPI is essential to repair DNA double strand breaks (DBSs) through the homologous recombination (HR).According to our working hypothesis, a RAD51-BRCA2 PPI inhibitor would chemically mimic the enhanced sensitivity to Olaparib observed in BRCA2-defective tumors, leading to a synthetic lethal effect (Figure 1A). [2] The RAD51-BRCA2 complex is mediated by two critical "hotspots" on RAD51 surface, zone I and II, which can lodge eight highly conserved BRCA2 motifs. [3] These two pockets make the RAD51-BRCA2 interaction suitable for a structure-based design of PPI small molecule disruptors. Herein, we focused on zone II, which has also proved to be crucial in RAD51's mechanism of action. [3] To date no inhibitors targeting zone II have been reported. Through a virtual screening campaign, we identified a dihydroquinolone pyrazoline-based molecule **1** as promising hit compound, which showed an inhibitory activity of RAD51-BRCA2 PPI in the competitive ELISA assay (EC_{50}= 16 ± 4 μM). [2,3] To discover more effective compounds and depict general structure-activity relationship (SAR) studies, we explored the chemical space around **1** by optimizing a general synthetic strategy and building a library that contains a variety of aromatic substitutions (green region) in combination with modifications of the acyl chain moiety (red region) (Figure 1B). [2] SAR efforts yielded **2** with the desired biological profile. As expected, **2** proved to disrupt the RAD51-BRCA2 PPI, inhibiting HR in pancreatic cancer cell line BxPC-3 and reproducing the paradigm of synthetic lethality in combination with Olaparib (Figure 1B). [2] These results make the fully small- molecule-induced synthetic lethality a suitable paradigm for the discovery of an innovative anticancer therapeutics.

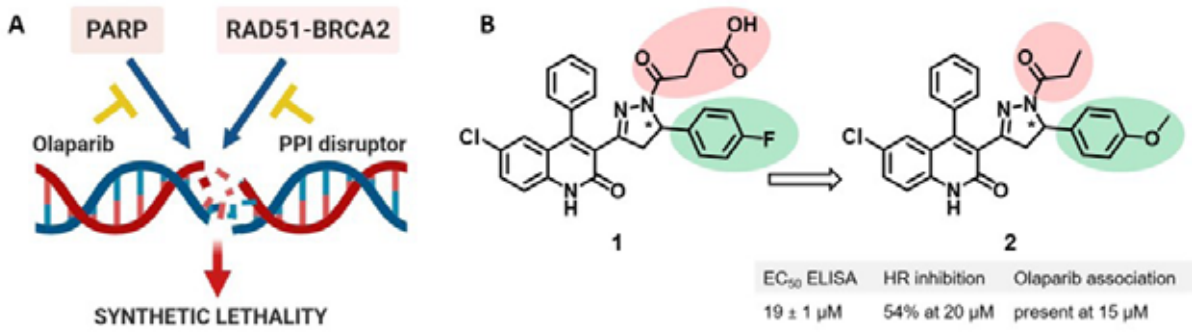

Figure 1. A) Fully small molecule-induced synthetic lethality concept; B) Identification of compound **2**.

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF POSITIONAL DERIVATIVES OF A SERIES OF N-PYRIDINYLCARBOXAMIDES AS POTENTIAL ANTITUBERCULOTICS

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Among infectious diseases, tuberculosis (TB) caused by bacillus *Mycobacterium tuberculosis*, is the leading cause of death worldwide[1]. The available treatment for TB is losing its efficacy due to the increasing incidence of antimicrobial resistance, and thus newer agents are urgently needed. A series of *N*-(pyridinyl)carboxamides was designed, prepared, and evaluated as potential antimycobacterials. Compounds to be presented are based on positional derivatives of picolinic acid linked to 4-aminosalicylic acid by amidic bond. 4-aminosalicylic acid is a second line antitubercular, and due to its low cost, it is still widely used in third-world countries[2]. Compounds were tested in vitro for biological activity against selected strains of *Mycobacterium* (slow growing *M. tuberculosis* H37Rv, *M. kansasii*, *M. avium*, fast growing *M. tuberculosis* H37Ra, *M. smegmatis, M. aurum, M. marinum*). The minimum inhibitory concentration (MIC) for tested mycobacterial strains was determined for all tested compounds beside isoniazid, ciprofloxacin and rifampicin as a reference standard drug. Additionally, we tested title compounds for antibacterial and antifungal activity, cytotoxicity against human HepG2 liver carcinoma cell line and further screening is still in process. Results of the biological testing and structure activity relationships are discussed in the presentation.

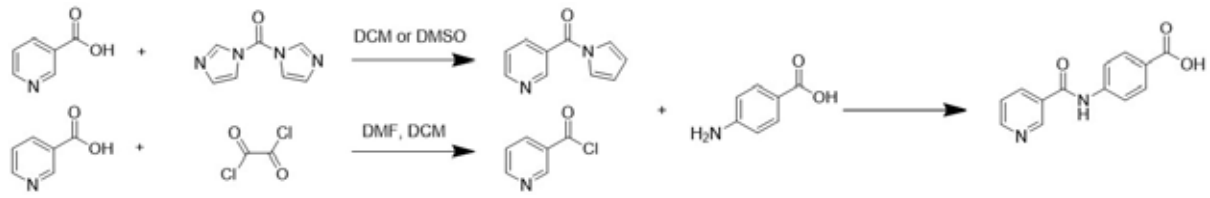

Figure 1. Synthesis scheme

The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (SVV 260 547) and by Grant Agency of Charles University (project C-C3/1572317).

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HIT OPTIMIZATION FOR THE DISCOVERY OF NOVEL RNF5 INHIBITORS AS CORRECTORS OF THE MUTANT CFTR CHLORIDE CHANNEL IN CYSTIC FIBROSIS

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In cystic fibrosis (CF), deletion of phenylalanine 508 (F508del) in the CF transmembrane conductance regulator (CFTR) anion channel is associated to misfolding and defective gating of the mutant protein. Among the known proteins involved in its processing one of the most promising drug target is the ubiquitin ligase RNF5, which promotes F508del-CFTR degradation. Recently, our group reported that genetically suppressing *in vivo* RNF5 increases CFTR activity in intestinal epithelial cells, suggesting that RNF5 inhibition could trigger F508del-CFTR rescue.[1] Therefore, through computational methods, we discovered **inh-2**, a drug-like small molecule that efficiently inhibits RNF5 ligase activity (Fig. - A). Evaluation of **inh-2** efficacy on CFTR rescue showed that **inh-2** decreased ubiquitylation of mutant CFTR and increased chloride current in human primary bronchial epithelia.[2]

Based on the promising biological results obtained with **inh-2**, we focused on the design and synthesis of a library of **inh-2** analogues. The series of new derivatives show structural variants introduced around the central 1,2,4-thiadiazol-5-ylidene core (Fig. - B), in order to explore the structure activity relationship of this class of compounds. As a primary screen, the new analogues were tested for their corrector activity in CFBE41o- bronchial epithelial cells, by using the microfluorimetric YFP assay.

Some of the new analogues displayed a greater efficacy than **inh-2**, demonstrating that the 1,2,4-thiadiazolylidene scaffold is a versatile architecture for the identification of RNF5 inhibitors, able to rescue F508del-CFTR trafficking defect in human bronchial epithelia. These finding validate RNF5 as a drug target for CF and provide evidences to support its druggability.

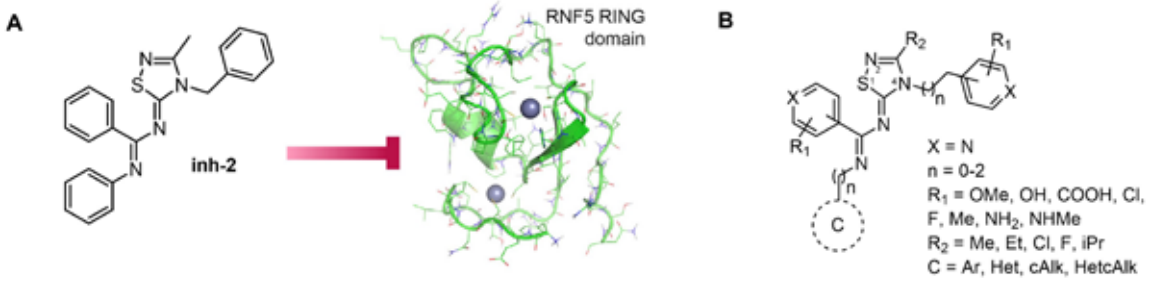

Figure: A) inh-2 stucture and activity; B) library of planned **inh-2** analogues.

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TOWARDS THE FORMAL SYNTHESIS OF AMPHIDINOLIDE K

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The symbiont dinoflagellate Amphidinium is an incredible biological manufacturing unit. According to our knowledge, 29 interesting molecules have been isolated from Amphidinium sp. In 1989, Kobayashi [1] discovered at least 10 Amphidinolides (A-J) showing cytotoxic activities against murine leukemia P388, L1210 cells and human epidermoid carcinoma KB cells. Four years later, Kobayashi [2 & 3] isolated a new molecule, Amphidinolide K. Three total syntheses of Amphidinolide K have been reported by Williams [4], Lee [5] and Vilarrasa [6]. One of the big challenge is the exo-methylene THF pattern. We became interested in the synthesis after Dr T. Xhurdebise [7] discovered a fortuitous ring contraction and exemplified it. Combined with the ene-IMSC sequence, this methodology represents a powerful tool to synthesize the THF ring of Amphidinolide K.

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DESIGN AND SYNTHESIS OF NEW RNA LIGANDS FOR THERAPEUTIC APPLICATIONS

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The design and synthesis of nucleic acids ligands recently became a major issue in the medicinal chemistry field of research. In particular, RNA is one of the most intriguing and promising biological target for the discovery of innovative drugs in a large number of pathologies[1]. Various biologically relevant RNAs that could serve as drug targets have already been identified such as mammalian microRNA (for anticancer therapies), bacterial RNA (discovery of new antibiotics) and viral RNAs[2]. Given that some of the reported RNA ligands still lack selectivity, large efforts to develop specific binders recently succeded with the FDA approval of Risdiplam (Evrysdi™, Roche) as a mRNA splicing modifier against spinal muscular atrophy[3]. This, together with a large number of marketed antibiotics able to bind prokaryotic ribosomal RNA inhibiting protein synthesis in bacteria, proves that RNA binding with specific ligands could represent an extremely promising therapeutic strategy.

The aim of the present work is to take advantage of the covalent binding mode of some DNA alkylating agents, used in the treatment of several cancers, for the design of novel RNA ligands bearing the ability to bind the target in a covalent manner. With this strategy, we aim to design more selective and efficient ligands composed of (a) a well-kown RNA ligand to interact with the target and (b) a covalent DNA ligand to strongly bind to the target (*Figure 1*). In this context, we decided to target both oncogenic miRNAs for anticancer applications and procaryotic RNAs for innovative antibacterial approaches. The stability and biological activity of the synthezised compounds will be evaluated against these different types of RNA targets.

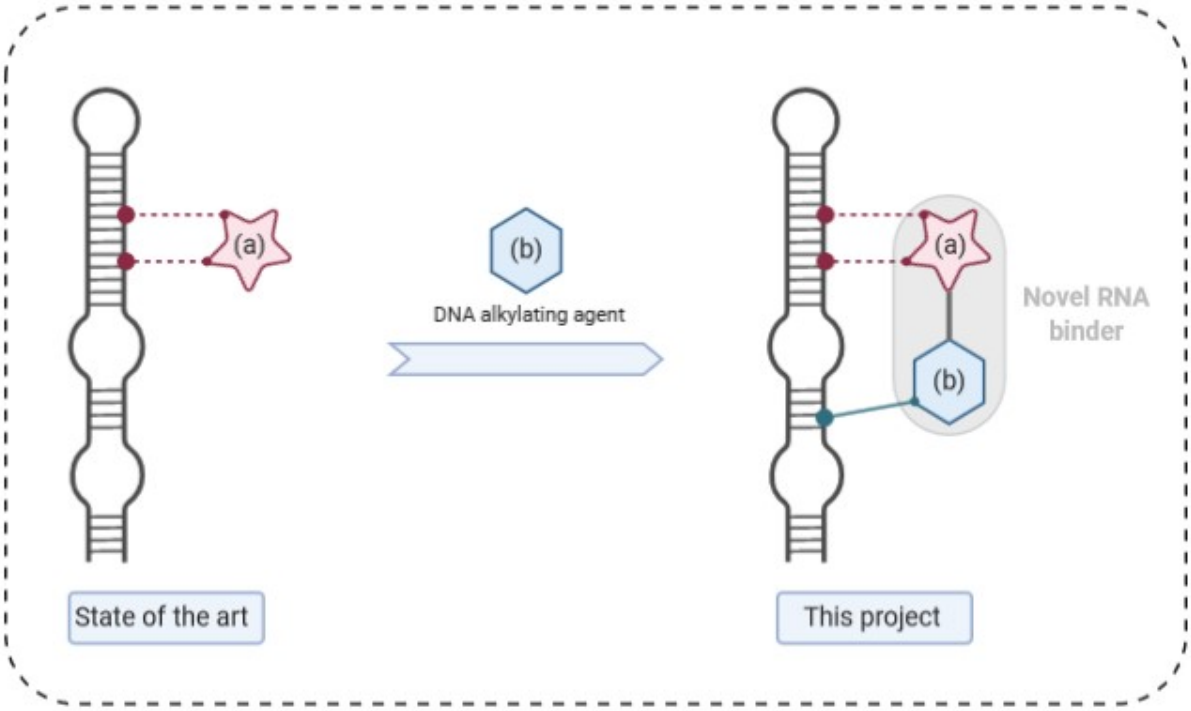

Figure 1. Design of novel RNA binders.

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NOVEL 3-SUBSTITUTED PYRAZINE-2-CARBOXAMIDES AS POTENTIAL ANTIMYCOBACTERIAL AGENTS

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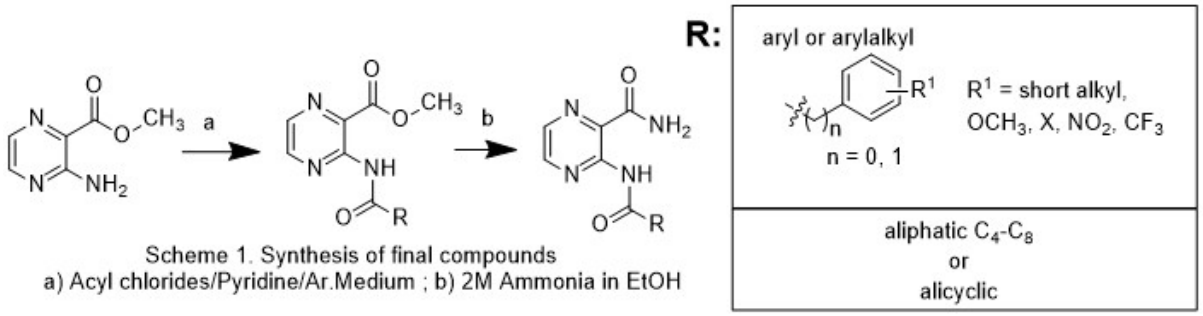
Tuberculosis (TB) remains among the WHO top ten causes of deaths despite available treatments[1]. As a part of our ongoing research on pyrazinamide (a first line antitubercular) derivatives, we report the design, synthesis, and biological evaluation of novel 3-substituted derivatives of pyrazine-2-carboxamides. A total of 29 compounds were prepared according to Scheme 1 and evaluated for their *in vitro* activity against the avirulent *Mtb* H37Ra*, M. smegmatis*, and *M. aurum* strains. Those with promising activities were further advanced into *in vitro* screening against the virulent *Mtb* H37Rv strain. Final compounds were also evaluated for their *in vitro* antibacterial and antifungal activities, beside cytotoxicty on HepG2 cell line. Obtained results will be discussed in the poster. As a complementary investigation, title compounds will be also studied as potential inhibitors of (human) prolyl-tRNA synthetase based on their structural similaries to the confirmed inhibitors reported in the literature[2].



Scheme 1. Synthesis of final compounds
a) Acyl chlorides/Pyridine/Ar.Medium ; b) 2M Ammonia in EtOH

The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (SVV 260 547) and by CELSA—Project title: Structure-based design of new antitubercular medicines—KU Leuven (Arthur Van Aerschot)—Charles University in Prague (Martin Doležal).

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APPROACH TO THE TOTAL SYNTHESIS OF GULMIRECIN B

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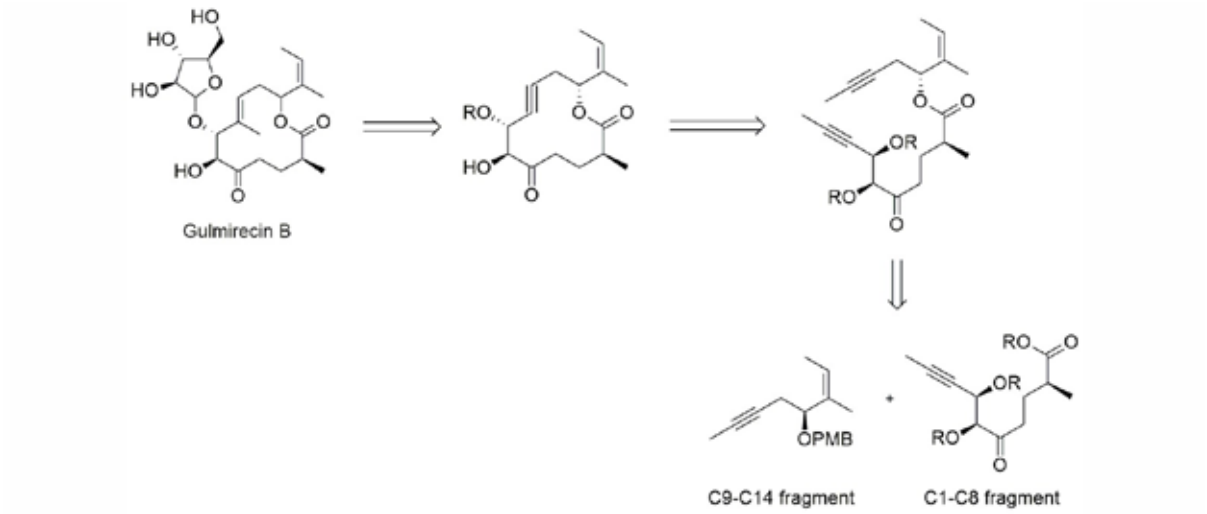
In recent times, the rise of multiresistant bacteria has become a challenging problem, leading to numerous deaths especially in hospitals[1]. Therefore, it is crucial to keep an eye on fundamental antibiotic research and further development of already known antibiotics.

The Gulmirecins A and B are twelve-membered macrolides, which were isolated in 2013 out of the myxobacteria *Pyxidicoccus fallax* HKI 727 by Nett *et al*.[2]. These structures are closely related to the Disciformycins, which were discovered in the same year by Müller *et al*.[3]. The Gulmirecins, as well as the Disciformycins, were tested for their antimicrobial activity and some of them were considered as active against MRSA (methicillin-resistant staphylococcus *aureus*)[4]. Interestingly some of them turned out as active against mutants that already developed resistance against other macrolide antibiotics. This led to the assumption that their activity is based on different biological pathways. Kirschning and co-workers hypothesize that Gul A and B, as well as Dis A and B, might be products of different stages out of the same biosynthesis[5].

Apart from their biological activity, they are interesting targets for total synthesis due to their structural motifs. Up to this point, there are four publications from Fürstner[6], Kirschning[5], Ichikawa[7] and Maier *et al*.[8], which are addressing the total synthesis of these macrolides.



The centre of this work is Gulmirecin B which contains two alkene functions and four stereocentres in the macrolide motif. In our current approach, the core structure is build up from two major fragments. The C1-C8 fragment and a C9-C14 fragment. The first fragment contains the C6-C7 diol originated from D-tartrate whereas the second part is synthesized from D-malic acid.

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REVEALING A SIGNIFICANT CONFORMATIONAL SWITCH OF H12 IN THE PROGESTERONE RECEPTOR AND ESTROGEN RELATED RECEPTOR α TROUGH MOLECULAR DYNAMICS SIMULATION

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Nuclear receptors (NRs) are highly pursued druggable targets in biomedical research since they play a key role in gene regulation and are involved in a wide range of severe pathologies such as cancer, inflammation and cardiovascular diseases.[1] One of the main features of NRs is their inherent structural flexibility that makes them challenging targets for drug discovery especially when performing structure-based drug design (SBDD) campaigns.[2] In particular, helix 12 (H12), part of the Ligand Binding Domain (LBD), undergoes different conformational rearrangements upon ligand binding, leading to different biological outcomes trough the recruitment of specific co-regulators.[3] Investigating the structural dynamics of NRs is crucial to elucidate ligand-induced fits and to reproduce a pool of alternative receptor conformations to facilitate the rational design of drug leads. Here we report the use of Molecular Dynamics (MD) simulations to reveal the dynamic behaviour of the progesterone receptor (PR) and estrogen related receptor α (ERRα). Using plain extensive MD simulations, we show significant antagonist/agonist and agonist/antagonist transitions of H12 in the apo form of PR and ERRα, respectively. In contrast, no transition is observed when simulating the opposite starting conformations. Taken together, these findings suggest the agonist-like conformation of PR and the antagonist-like conformation of ERRα as the most stable LBD arrangements in absence of both ligand and co-regulator. At the same time, these results can be used for the development of specific structure-based strategies targeting PR and ERRα and emphasize the importance of MD to investigate large conformational transitions within biomolecules.

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IDENTIFICATION OF NOVEL PHYTOCANNABINOIDS ISOLATED FROM A MEDICAL CANNABIS SATIVA VARIETY: CANNABIDIBUTOL (CBDB) AND Δ9-TETRAHYDROCANNABUTOL (Δ9-TCHB)

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Cannabis sativa has been for a long time a neglected plant as it is the most widely spread illicit drug worldwide, especially among young adults. However, in the last decade, a renewed interest on Cannabis has arose in the scientific community, especially for its implication in the treatment of several pathologies including glaucoma and epilepsy, definitely becoming one of the most studied plants. Cannabis is the only plant able to produce a peculiar class of organic molecules called phytocannabinoids. The two most important and studied phytocannabinoids are undoubtedly cannabidiol (CBD), a non-psychotropic compound, but with other pharmacological properties including anti-inflammatory, anti-oxidant and anti-convulsant activity, and Δ^9-tetrahydrocannabinol (Δ^9-THC) which instead possesses psychotropic activity and it is responsible for the recreative use of hemp. Beside CBD and THC, at present, almost 150 phytocannabinoids have been detected in the cannabis plant, although few have been isolated and studied.

However, the analysis performed by our research group on a medicinal cannabis variety, the Italian FM2, provided by the Military Chemical Pharmaceutical Institute in Florence by means of UHPLC-HESI-Orbitrap, allowed us to identify some still unknown phytocannabinoids. In particular, the butyl homologues of CBD (called CBDB) and Δ^9-THC (called Δ^9-THCB) were isolated by semipreparative HPLC purification.

For the first time, their absolute configurations were assigned, and their chemical and spectroscopic properties were thoroughly characterized and compared to those of authentic standards obtained via stereoselective synthesis.[1,2] Extensive pharmaceutic studies were performed on the two new identified THCs. The biological results obtained in the *in vitro* binding assay indicated an affinity for CB1 receptor comparable (K_i=15 nM, for Δ^9-THCB)[2] to the one reported for Δ^9-THC in the literature. Docking studies confirmed the importance of the length of the alkyl chain on the resorcinyl moiety for CB1 binding affinity.

In vivo formalin test was performed on Δ^9-THCB revealing potential analgesic and anti-inflammatory properties.[2] The tetrad test in mice showed a partial agonistic activity of Δ^9-THCB toward the CB1 receptor. Ongoing studies are devoted to the investigation of the pharmacological activity of CBDB and to expand that of Δ^9-THCB. These new identified phytocannabinoids are present in traces and therefore they do not represent a threat for the human health. However, these remarkable works highlight how much there is to still learn about the properties of cannabis paving the way to pharmacologists, toxicologists, and clinicians to new therapeutic substances and to correlate the observed biological effects with the chemical composition of the different cannabis varieties employed.

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TOWARDS MEDICINAL MECHANOCHEMISTRY APPROACH FOR AN EFFICIENT AND SUSTAINABLE SYNTHESIS OF A POTENT 5-HT7 RECEPTOR ANTAGONIST

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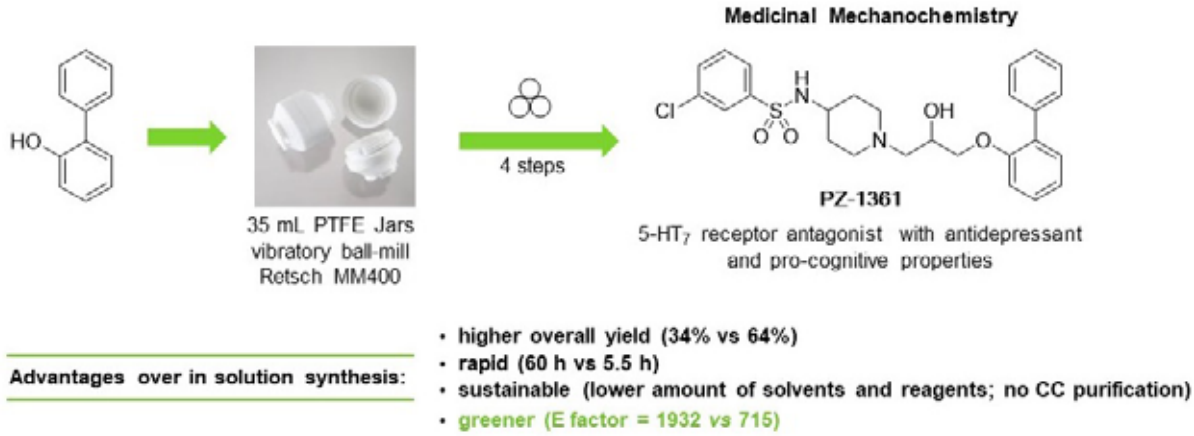
Recently, mechanochemical synthesis has been recognized as an innovative and efficient methodology [1], and has a hot topic of study in both academic and industrial research. The primary driving force underlying the rediscovery of mechanochemistry is green chemistry,[2] in particular the need of chemical and pharmaceutical industries for the development of more sustainable synthetic protocols characterized by energy efficiency of chemical transformations and reduction of solvent use. The use of such approaches offers additional advantages of mechanosynthesis over classical organic chemistry, in terms of excellent selectivity and the possibility to perform previously unknown reactions. Interestingly, an increasing number of mechanochemical procedures for generating pharmaceutically relevant fragments and functionalities led to coining the term “medicinal mechanochemistry”[3].



Here, an efficient and sustainable mechanochemical procedure was developed to obtain **PZ-1361**, a potent and selective 5-HT$_7$ receptor antagonist, with significant antidepressant properties in rodents [4]. The elaborated protocol offered several advantages over classical thermal methods in solution, including improvement of the overall yield (from 34% to 64%), reduction of reaction time (from 60 h to 5.5 h), limitation of the use of toxic solvents and the formation of by-products. The versatility of the protocol was additionally confirmed by introducing diversification at the aryloxy fragment, central amine core, and the benzenesulfonamide moiety. This approach represents a rare example of organic synthesis of biologically active compounds exclusively performed using mechanochemical and solid/gas reactions [5].

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SYNTHESIS OF 3,4-DIARYL-5-METHYLISOXAZOLES WITH POTENT ANTIPROLIFERATIVE ACTIVITY AGAINST A PANEL OF HUMAN LIVER AND BREAST CANCER CELL LINES

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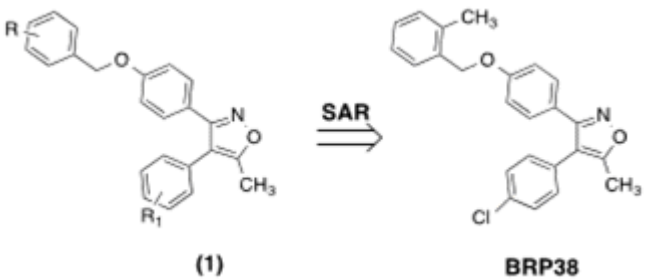
Isoxazole ring is a popular scaffold widely used for development of novel anticancer therapeutics, and among them, *o*-diarylisoxazoles has recently gained attention as *cis*-restricted analogues of combretastatin A-4, which is a highly potent natural cytostatic agent isolated from the bark of African willow tree *Combretum caffrum*. In this presentation, we report the synthesis of 3,4-diaryl-5-methyl isoxazoles (**1**) by 1,3-dipolar cycloaddition of the aryl nitrile oxides to the enolates of phenyl acetone derivatives. The generation of aryl nitrile oxides was achieved from chlorobenzaldoxime derivatives, which were obtained in situ by reaction of appropriate arylhydroxamic acids with N-chlorosuccinimide. The orientation of aryl rings in relation to isoxazole heterocycle was confirmed by X-ray crystallography. Targeted compounds were initially screened for their antiproliferative activity against selected cancer cell lines including liver (Huh7), breast (MCF7) and colon (HCT116) to derive structure-activity relationships. Among them, the compound BRP38 showed the most potent cytotoxic bioactivity to all tested cell lines (IC_{50} = 1.2-3.4 μM) and was further evaluated against a panel of human liver (HepG2, Snu475, Hep3B, Focus and Mahlavu) and breast (MCF12A, MDA-MB231, MDA-MB468, SKBR3, ZR75) cancer cells. BRP38 showed potent cytotoxicity with IC_{50} values in the range of 1.74-7.64 μM. The time dependent bioactivity of BRP38 was further analyzed with real time RT-CES systems and the IC_{50}values were in parallel with NCI-SRB end-point assay. For exploration of the mechanistic aspects of the observed bioactivity with BRP38, further molecular cellular experiments were carried out. First the cellular pathways were investigated with western blot analysis and band intensities due to protein expression and post translational modification were compared with vehicle control. In this study, we showed an increase in the cleavage of apoptotic proteins PARP and Caspase-3 as well as increased GSK-3-beta expression in BRP38 treated Mahlavu cells. The activation of apoptotic pathway was also supported by Hoechst staining of the condensed chromatins, nuclear fragmentation and the apoptotic bodies upon BRP38 treatment. Furthermore, we demonstrated an increase in the activation of stress activated protein p38 and cell cycle regulator protein p21 in BRP38 treated liver cancer cells. The increment in another stress activated protein, SAPK/JNK was shown in breast cancer cells (MDA-MB-231). Additionally, the effect of BRP38 on the cell cycle was analyzed with flow cytometer and the data indicated that BRP38 induced G2 phase arrest in Huh7, Mahlavu, MCF7 and ZR75 cancer cells. The number of subG1 detained apoptotic cells were also increased in BRP38 treated liver and breast cancer cells. To conclude, BRP38 was found to be a potential anticancer agent for further development, which acts through induction of cell stress and apoptotic pathways ultimately leading to cell death (This study is supported by TUBITAK Research Grant 215S015).



(1) BRP38

VICINAL DIARYL HETEROCYCLIC COMPOUNDS WITH POTENTIAL ANTICANCER ACTIVITY

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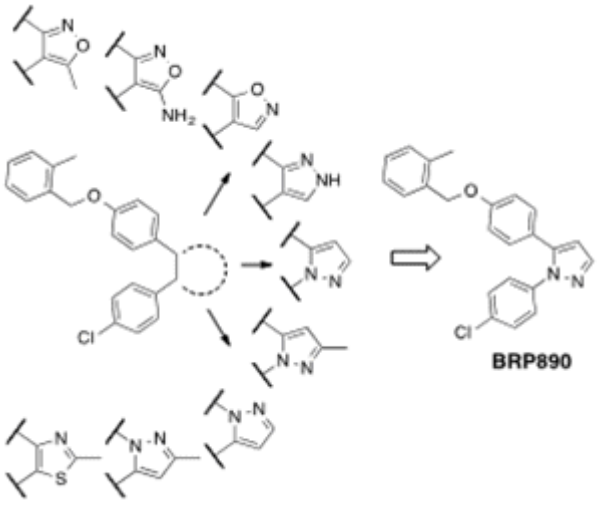
Vicinal diaryl heterocyclic system, a privileged scaffold, is present in a variety of compounds having diverse biological activities, and various vicinal diaryl heterocyclic compounds have been reported showing potent anticancer activities.

In light of the promising data, we have recently developed 3,4-diaryl-5-methylisoxazole derivatives exemplified with 4-(4-chlorophenyl)-3-methyl-5-{4-[(2-methylphenyl)methoxy]phenyl}-1,2-oxazole (BRP445) with significant anticancer activity against liver and breast cancer cell lines with IC_{50} values in the range of 1.74 to 7.64 μM.

In an effort to develop new derivatives with improved anticancer activity, a closely related congeneric series to BRP445 by replacing the central isoxazole ring with isosteric five-membered heterocycles was prepared and screened for their anticancer potential. As a result of the observed SAR, the 1,5-diarylpyrazole congener (BRP890) was the most potent among the five-membered heterocycles prepared (IC_{50}= 0.77-1.75 μM). In this presentation, we will present the in vitro analysis of the nature of cell death induced with BRP890 in liver and breast cancer cells as well as its in vivo efficacy in human liver xenografts (This study is supported by TUBITAK Research Grant 215S015).



LIGAND IDENTIFICATION FROM NP-INSPIRED COLLECTIONS USING AFFINITY SELECTION - MASS SPECTROMETRY

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High throughput screening (HTS) has been the main driver for hit generation since its introduction in the mid-1980s. Pharma companies have routinely relied upon screening collections of 0.5-3 million compounds, at a throughput of 1 million compounds/day.[1] As pressure to reduce R&D costs is dramatically increasing, alternative paradigms are slowly emerging. Notably Fragment Based Drug Discovery (FBDD)[2] and DNA-Encoded Library Technology (DELT) [3] have opened up new possibilities to address targets that failed to deliver using HTS.

Affinity screening is becoming particularly attractive due to the recent emergence of PROTACs[4] resulting in a quest for selective binders versus functional hits. Yet, the DELT approach suffers from several drawbacks, namely resource-intensive deconvolution and sometimes difficult resynthesis of identified ligands due to the reaction conditions initially used for DNA library generation.

Complementary to this approach, **affinity selection mass spectrometry**[5] **(AS-MS)** is a screening method that enables large collections to be assayed as defined mixtures of compounds. The biomolecular target being screened needs not be labeled and compounds can be tested without need for tags, as each compound is self-encoded by its exact mass. AS-MS is a binding assay and allows the identification of ligands as hit structures irrespective of their functional effects. We will present in this poster the attractiveness and the results of combining AS-MS technology with a new chemical space inspired by natural products (NP) to tackle challenging targets in drug discovery.

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HOMOLOGY MODELLING OF THE STRUCTURE OF IONOTROPIC GLUTAMATE RECEPTORS

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The significance of (*S*)-glutamate as the main neurotransmitter in the central nervous system (CNS) has been thoroughly investigated since the 1960s and about 20 types of glutamic acid receptors have been described and classified. The group of receptors associated with Na^+, K^+ and Ca^{2+} channels includes NMDAR, AMPAR and kainate receptors (KAR) subfamilies, named after their major selective and exogenous agonists. Intense interest in the pharmacology and pathophysiology of the NMDA and AMPA receptors over the past years has revealed their important roles in the development of nervous system diseases (among others pain and epilepsy). On the contrary, the physiological function of KA receptors is still an open question and only limited data concerning its importance in CNS is available. Recent studies confirm that KARs are involved in short- and long- term synaptic plasticity and have an established modulatory role in synaptic transmission.

The present project is the continuation of recently published results of studies on the new series of quinoxaline-2,3-dione derivatives acting as AMPAR/KAR antagonists with structural modifications within the *N* 1-amide moiety as well as 6-positions of the bicyclic core[1,2] The understanding of the kainate receptors function has increased with appearance of X-ray data for the ligand binding domain (LBD) of the individual KAR subunits in complex with various ligands bound. Detailed information derived from crystallographic studies allowed to investigate activation mechanisms and provided an insights for the design of the compounds with improved affinity and selectivity. Most of the reported data concerned GluK1-selective ligands but currently, there are no available crystal structures of the GluK2 and GluK3-LBD with bound antagonists that could aid the design of new *N*1-benzamido substituted quinoxalinedione antagonists aiming for selectivity at the GluK3 subtypes.

Based on X-ray GluK1-LBD structure templates in complex with antagonists, the GluK3-LBD model was obtained. All this allowed to indicate the amino acids responsible for the binding selectivity and also enabled to explain the cause of the activity or lack of it for a group of ligands.

This work was supported by Jagiellonian University Medical College (*grant to. N42/DBS/000113 and N42/DBS/000042).*

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IN SILICO ANALYSIS OF NEWLY SYNTHESIZED 4-HYDROXYCOUMARIN-CARBONITRILE DERIVATIVES AS POTENTIALS INHIBITORS OF ACETYLCHOLINESTERASE

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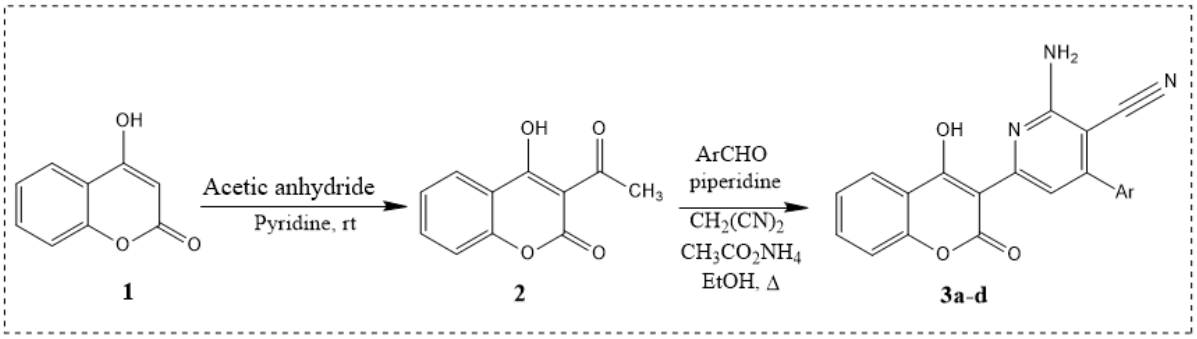
4-Hydroxycoumarins have evoked a great deal of interest due to their biological properties and characteristic conjugated molecular architecture.[1]

The existing data on the bioactivity of natural and synthetic coumarins is very promising, thus these heterocyclic compounds can be considered as an attractive scaffold for drug design and development. Many of them display important pharmacological effects, including analgesic, anti-inflammatory anticholinesterase, antibacterial, antiviral, and anticancer properties.[2]

Using computer aided drug design and previous biological data from known hydroxycoumarins, we designed and synthesized a series, of novel 4-aryl-2-amino-6-(4-hydroxycoumarin-3-yl) pyridine-3-carbonitriles **3a-d** by the reaction of 3-acetyl-4-hydroxycoumarin with some aromatic aldehydes in ethanol in the presence of a catalytic amount of piperidine.

The Structures of all synthesized compounds were determined using spectroscopic methods including NMR.

A molecular docking study was carried out to evaluate the *in silico* anti-acetylcholinesterase potential of the synthesized compounds by using docking Tools.



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A DUAL-ACTING 5-HT6 RECEPTOR INVERSE AGONIST/MAO-B INHIBITOR WITH GLIOPROTECTIVE AND PROCOGNITIVE PROPERTIES

Vittorio Canale (1), Katarzyna Grychowska (1), Rafał Kurczab (2), Mateusz Ryng (2), Abdul Raheem Keeri (1), Grzegorz Satała (2), Agnieszka Olejarz-Maciej (1), Paulina Koczurkiewicz (1), Marcin Drop (1), Klaudia Blicharz (1), Kamil Piska (1), Elżbieta Pękala (1), Paulina Janiszewska (1), Martyna Krawczyk (2), Maria Walczak (1), Severine Chaumont-Dubel (3), Andrzej J. Bojarski (2), Philippe Marin (3), Piotr Popik (2), Paweł Zajdel (1)

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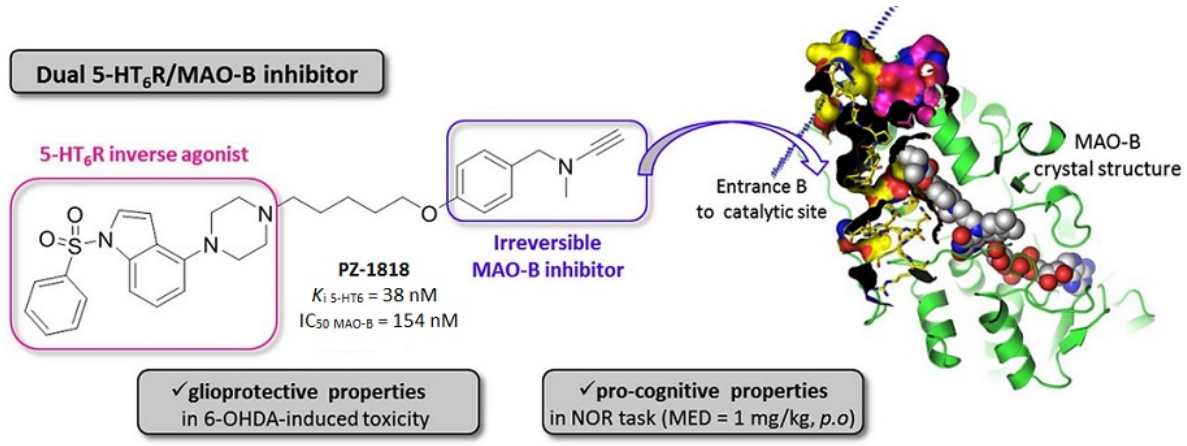
The complex etiology of Alzheimer's disease has initiated a quest for multi-target ligands to address the multifactorial causes of this neurodegenerative disorder.[1] Compounds modulating 5-HT$_6$R transmission have emerged as promising treatment of cognitive deficits related to neurodegenerative and psychiatric disorders.[2,3] Additionally, monoaminooxydase-B (MAO-B) inhibitors have been investigated as potential neuroprotective agents.[4] Based on these findings, we designed and synthesized a series of hybrids that link an (indol-4-yl)-piperazine fragment, a well-established core of 5-HT$_6$R antagonists, with frameworks of known MAO-B inhibitors, connected through an alkylene spacer.



The study identified compound **PZ-1818** which acts as an inverse agonist of 5-HT$_6$R at G$_s$ signaling and an irreversible MAO-B inhibitor. Compound **PZ-1818** showed moderate metabolic stability in rat microsomal assay, artificial membrane permeability, no hepatotoxicity, and it was well distributed to the brain. Moreover, compound **PZ-1818** reduced the gliotoxic effect of 6-OHDA in C8-D1A astrocytes in two complementary assays (MTT and LDH) assessing cell survival. Importantly, this effect was not observed after treatment with intepirdine (5-HT$_6$R antagonist) and MAO-B inhibitor-selegiline. Finally, compound **PZ-1818** (MED = 1 mg/kg, *p.o.*) fully reversed memory deficits in the NOR task induced by scopolamine in rats. These properties highlight **PZ-1818** as an original example of dual 5-HT$_6$R/MAO-B modulator and as an interesting prototype in the development of new treatment strategies for AD.[5]

Study was financially supported by National Science Centre, Poland, grant no 2016/21/B/NZ7/01742.

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MOLECULAR INSIGHTS INTO THE INHIBITORY ACTIVITY OF PANICEIN A HYDROQUINONE (PAH) FOR HEDGEHOG RECEPTOR PATCHED1

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In the context of Multi Drug Resistance (MDR), the overexpression of membrane multidrug transporters, namely ATP Binding Cassette (ABC) transporters, is one of the main reasons of failure in clinical cancer treatment. However, none of the proposed inhibitors of the major efflux systems has been approved by the Food and Drug Administration to date, due to severe side effect mainly related to the ubiquitous localization of these transporters [1]. The lack of efficient tumor specific targets is still one of the main issues in the fight against cancer.

The Hedgehog receptor Patched1 (PTCH1), part of the Hedgehog signaling pathway is involved in tissue development and differentiation in embryogenesis, but also in tissue homeostasis in adults and in cancer. PTCH1 has recently been shown to transport structurally and mechanistically different chemotherapeutics (doxorubicin, vemurafenib, cisplatin) out of cancer cells thus contributing to MDR to cancer treatment. A screening of natural compounds purified from marine sponges led to the identification of the first Patched1 efflux inhibitor, namely panicein A hydroquinone (PAH) [2]. This compound was shown to increase doxorubicin cytotoxicity *in vitro* and vemurafenib cytotoxicity *in vitro* and *in vivo* in melanoma cells [3]. Due to PTCH1 overexpression in different types of cancer and to the characteristic pH gradient in tumor environment which is thought to be the driving force for drug efflux, PTCH1 inhibition is highly selective for cancer cells.

Molecular insights into the mechanism by which PAH binds to PTCH1 and inhibits sterols and chemotherapeutics transport are addressed in this study by means of different computational techniques. We first performed a thorough characterization and druggability analysis of the main putative substrate binding pockets known from available cryo-electron microscopy structures. Then, conformational analysis and dynamical descriptors of the active (PAH and related compounds) and inactive (PA and related compounds) analogues were extracted from microsecond-long all-atom molecular dynamics simulations in water solution. Finally, a binding mode prediction through a blind ensemble docking methodology enabled to rationalize the interaction between PTCH1 and PAH and derivatives in terms of their intrinsic physico-chemical properties. The obtained results can be useful in future drug design in order to synthesize compounds that will more potently inhibit PTCH1 drug efflux activity and, in combination with standard treatment, increase cancer cells death.

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ABIETANE DITERPENOIDS ISOLATED FROM PLECTRANTHUS SPP. INDUCE CANCER CELL DEATH VIA APOPTOSIS

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Medicinal plants have been used for centuries to treat several ailments. *Plectranthus* genus (Lamiaceae) has a diversity of species that allowed the obtention of compounds with promising biological activities [1]. An example of such is the cytotoxic diterpenoid parvifloron D (Parv) (Figure 1), isolated from *Plectranthus ecklonii* Benth. [2]. Likewise, the royleanones 7β,6β-Dihydroxyroyleanone (Diroy) and 7α-acetoxy-6β-hydroxyroyleanone (Roy) (Figure 1), obtained from in *P. madagascariensis* (Pers.) Benth., displayed notable bioactivities [3]. Furthermore, the abietane diterpene 6,7-dehydroroyleanone (Deroy) (Figure 1), isolated from *P. madagascariensis* essential oil, also presented an interesting cytotoxic effect [4].

In the present study, the cytotoxic properties of four abietanes (Roy, Deroy, Diroy, and Parv) are investigated in acute lymphocytic leukemia (CCRF-CEM) and lung adenocarcinoma cell lines (A549). The tested compounds revealed cytotoxic effects against CCRF-CEM and A549 cell lines. Additionally, all tested compounds showed the ability to change the level of pro- and anti-apoptotic genes, and consequently, induce apoptosis. Roy and Parv demonstrated the strongest activity in both human cancer cell lines, changing the permeability mitochondrial membrane potential and reactive oxygen species (ROS) levels. Moreover, Parv is responsible for the increase of mtDNA damage in both regions (ND1 and ND5) of CCRF-CEM cells, while Roy produced an intensification in the nDNA damage in the HPRT1 region of A549 cells. Therefore, these abietane diterpenoids could be used in the future as potential natural chemotherapeutic agents.

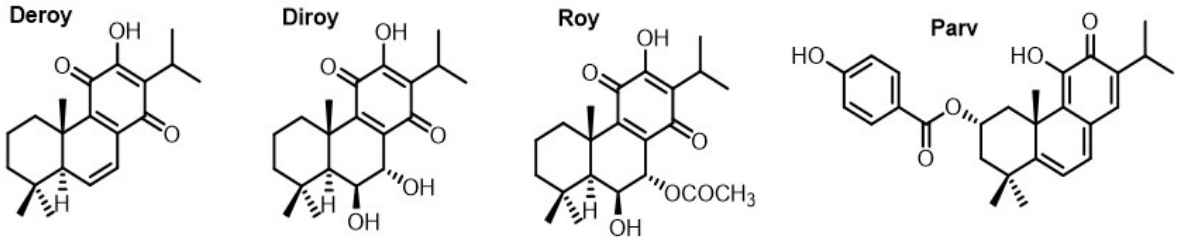

Figure 1 - Abietane diterpenes isolated from *P. madagacariensis* and *P. ecklonii.*

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IN SILICO IDENTIFICATION OF CROSS-CLASS INHIBITORS AGAINST CLINICALLY RELEVANT BETA-LACTAMASES

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Antibiotic resistance is worryingly spreading worldwide, especially among the most dreaded ESKAPE bacteria[1]. A main resistance mechanism involves the production of beta lactamases (BLs, i.e. enzymes able to degrade beta lactam antibiotics), which are conventionally divided in four classes (A, B, C, or D)[2]. In particular, class A, C and D enzymes use a catalytic serine to degrade beta lactam antibiotics, while class B enzymes exploit a zinc-based catalytic hydrolysis. As the road to new classes of antibiotics is long and increasingly problematic, the inhibition of BLs constitutes an effective strategy to potentiate or restore the activity of current antibiotics. In this work, we focused on finding inhibitors against five relevant BLs (class A CTX-M-15 and KPC-2, class B NDM-1 and VIM-2 MBLs, and the class C AmpC). A first *in silico* screening of a commercially available library selected a pool of promising candidates, which were purchased and consequently tested against clinically relevant strains of bacteria producing BLs. The results highlighted that most effective compounds share electron donor moieties. In particular, KPC-2 is preferentially inhibited by sulfonamide and tetrazole-based derivatives, NDM-1 by compounds bearing a thiol, a thiosemicarbazide or thiosemicarbazone moiety, while VIM-2 by triazole-containing molecules. Moreover, we found few inhibitors able to target more than one class of BLs, thus exerting a “broad-spectrum” inhibition against both serine-based and zinc-based hydrolysis. *In vitro* tests highlighted that compound **40** enhances imipenem activity against an NDM-1 producing *E. coli* clinical isolate. Finally, X-ray structures of the two most promising compounds in VIM-2 and NDM-1 gave important insights about the binding mode of triazole-thiol scaffolds.

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FLUOROGENIC PROBES FOR BACKGROUND-FREE IMAGING OF G-PROTEIN-COUPLED RECEPTORS IN LIVING MICE

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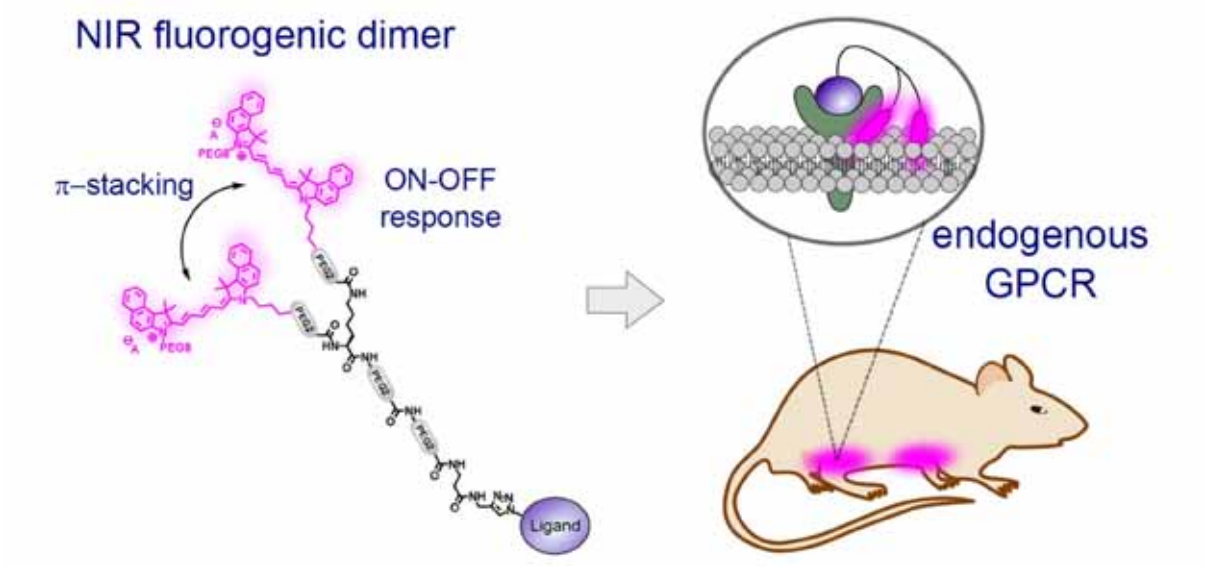
G-protein-coupled receptors (GPCRs) represent the largest family of transmembrane receptors in humans and the targets of more than 30% of all known drugs on the market. The ability to detect, measure and quantify the binding of ligands to these receptors both *in vitro* and *in vivo* represents key elements of the drug discovery process.

Taking advantages of the high sensitivity and the reduced environmental safety risk of fluorescence technics, we have recently developed potent and selective fluorescent probes to study the functional architecture of GPCRs, especially their ability to form heterodimers[1] but also to set up new receptor-selective high-throughput screening assays for drug discovery.[2]

For GPCR imaging, we have designed novel fluorogenic probes that turn-on their fluorescence after binding to their receptor enabling the unprecedented background-free imaging of GPCRs in living cells.[3] By introducing the new concept of fluorogenic dimers with polarity-sensitive folding,[4] we created a fluorogenic molecular imaging agent in the near-infrared which allowed for the first time the detection of the native oxytocin GPCR in living mouse.[5] This concept opens the way to the non-invasive and non-ionizing fluorescence cartography of GPCRs in living animals.



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SYNTHESIS, STRUCTURAL CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL SELENOESTERS

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Nowadays, cancer is one of the principal causes of morbi-mortality in the world. Its incidence continues increasing and the most frequent tumors are lung, breast, colon, prostate and stomach.

Despite of available treatments (surgery, radiotherapy, chemotherapy, immunotherapy, etc.) their efficacy is questionable. Therefore, an urgent necessity to develop novel therapies to increase and improve the live expectancy exists. In this context, selenium containing compounds emerge as a novel strategy. It has been observed that the chemical form, dose, and oxidative state of this chemical element are critical for its antitumoral activity. Even though several mechanisms of action are implicated, metabolism of selenocompounds is crucial, methylselenol metabolite being a key element for its activity.

Herein, 10 novel selenium derivatives are presented and characterized by infrared and nuclear magnetic resonance spectrometry (^{1}H, ^{13}C, ^{77}Se). In relation to their biological evaluation, these compounds were tested as cytotoxic agents in two different cancer cell lines: HT-29 (colon) and PANC-1 (pancreas). Furthermore, their radical scavenging capacity have been determined by the colorimetric assay of DPPH (1,1-diphenyl-2-picrylhydrazyl hydrate).

Among the newly synthesized compounds, methylselenoderivatives showed potent and selective activity towards colorectal cancer cell line (HT-29), with IC_{50} values ranging from 23 μM to 30 μM. Related with antioxidant capacity, 2 derivatives showed moderate activity in a time- and dose-dependent manner.

METHOD DEVELOPMENT FOR SIMULTANEOUS DETERMINATION OF VALSARTAN AND ATENOLOL IN NEWLY FORMULATED DOSAGE FORM

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Aim of this work was to develop the first simple, rapid, green, economical and selective HPLC method for simultaneous quantification of the cited drugs in their challenging binary mixture.
Materials and methods. This was accomplished under the following chromatographic conditions: HPLC column Discovery C18 (4.6 mm i.d. X 150 mm, 5 μm), column temperature 30 °C, flow rate 1.0 mL/min, mobile phase composed of 20 % acetonitrile, 80 % of 0.16 % ammonium acetate and 0.2 % of 1.5 M tetramethylammonium hydroxide (V/V) and signal monitoring at a wavelength of 225 nm and 237 nm.
Results. A conventional mixture of acetonitrile and 0.16 % ammonium acetate was tried in different ratios, but the drugs were not well separated. The shortest aliphatic chain cationic ion pair reagent tetramethylammonium hydroxide should not be exchanged with other type similar with this, like tetramethylammonium hydrogen sulfate, it did not work to our experiments. Increasing salt concentration, ammonium acetate, more than 0.2%, pushes the peak of atenolol closer to dead volume, which is negative. Atenolol in their methods for multicomponent mixtures elutes in dead volume, or when retained longer, much stronger, hydrophobic mobile phase should be used if valsartan should be seen in same chromatogram at dissent time. The 237 nm can be choosen as compromise signal for nearly equal peaks height with high sensitivity is not essential. The 225 nm signal shows much highter sensitivity for atenolol and less increase for valsartan peaks, which can be used when higher sensitivities will be essential. Linearity was examined and proven at different concentration levels in the range of working concentration of valsartan (16–96 mg/mL) and atenolol (20–120 mg/mL). The high value of recoveries obtained for valsartan and atenolol indicates that the proposed method was found to be accurate. The results of proposed method found to be an excellent green analysis with a score of 84.
Conclusion. A new fast, simple and green, but selective, accurate, precise and robust HPLC-UV method for simultaneous determination of valsartan and atenolol in newly formulated dosage form was developed and many possible variations of the same were suggested.

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NOVEL SELENOUREAS WITH CYTOTOXIC AND ANTIOXIDANT ACTIVITY

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Oxidative stress is a common pathogenic factor associated with aging processes and is involved in various diseases, including cancer. Several selenocompounds, such as ebselen and PBISe, have demonstrated to regulate the cell redox status. Hence, they are promising compounds for therapy and prevention of diseases directly related with reactive oxygen species (ROS) generation [1,2]. For these reasons, we consider that Se might be an important tool for the development of new drugs.

N,*N*′-disubstituted selenourea derivatives have been reported as nontoxic polyfunctional antioxidants with higher potency than sulfur or oxygen analogs [3]. In our research group, different acylselenourea derivatives have been proven as potent agents against different types of cancer, such as breast, colon or prostate [4].

Taking all these facts into account, herein we present the design, synthesis and cell growth inhibition and radical scavenging activity for 30 new *N*,*N*′-disubstituted selenoureas (Figure 1).

Eight compounds exhibited IC_{50} values lower than 10 μM in HTB-54 (lung), MCF-7 (breast) and several colon cancer cell lines. Additionally, at low doses most of the synthetized compounds showed outstanding radical scavenging capacity, higher than the references ascorbic acid or trolox. One compound was selected for the NCI-60 program with excellent results, and its cell cycle modulation capacity was also evaluated. Besides, flow-cytometry studies were performed to preliminary characterize the cell death mechanism induced by this compound.

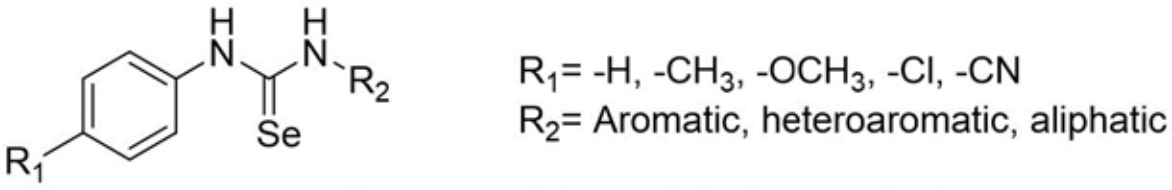

***Figure 1** : General structure of the N,N´-disubstituted selenoureas.*

To conclude, acylselenourea-based analogs might be consider a privileged frameworks to develop dual antioxidant and antitumor agents.

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DEVELOPMENT OF A SELENO-FRAMEWORK TO OBTAIN POTENT ANTIOXIDANT AGENTS

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Cancer is currently one of the major health problems of the human population and a prominent cause of death worldwide. It has been demonstrated that an excessive production of reactive oxygen species (ROS) is considered to be an important hallmark for carcinogenesis [1], and therefore, cellular oxidative stress for cancer treatment has driven the attention of the scientific community in recent years [2]. The introduction of a selenium atom (Se) in the structure of organic molecules has demonstrated to be a valid approximation in the design of novel chemotherapeutic agents with several biological effects, including antioxidant activity [3].

On the basis of these findings, and taking into account previous studies developed by our research group [4], we have designed 47 acylselenourea derivatives bonded to different carbocyclic and heterocyclic systems and modulated through *N*-substitution in the selenourea group with several aromatic rings functionalized with electron-withdrawing or electron-donating groups in '*para*' position.

Dose- and time-dependent radical scavenging activity for all the 47 organoselenic compounds were assessed using the *in vitro* DPPH and ABTS assays, showing that several derivatives present greater radical scavenging activity than ascorbic acid and trolox.

From the results obtained in these preliminary experiments, 4 hit compounds were selected to evaluate their protective effects against oxidative cell death induced by H_2O_2 on colon cancer cells. Compound **9c** achieved an increase on cell survival up to 3.6 fold, meaning that the acylselenourea scaffold could be a feasible frame to develop new agents with antioxidant properties.

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SYNTHESIS, ANTI-TUMOR AND ANTI-OXIDATIVE EVALUATION OF NOVEL SELENATED HYBRIDS

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Cancer includes a wide range of diseases, which are characterized by unregulated cell growth. These diseases are the second leading cause of death worldwide [1]. Cell resistance to conventional treatments and adverse side effects are the main challenges, making the research for new treatment approaches a priority [2]. Considering these facts, selenium-derived compounds have been developed as a new strategy for the treatment of cancer diseases [3]. Molecular hybridization strategy was used to synthesized two series of compounds, starting from fragments with well-known anti-tumor activity. Eight novel selenated hybrids were isolated. The cytotoxicity of the new compounds was evaluated in two tumor cell lines: **HT-29** (colon cancer) and **PANC-1** (pancreatic cancer). Optimal results were obtained for all the compounds in colon cancer, giving $\mathbf{IC_{50}}$ values close to 10 μM. Selectivity towards colon cell line was observed in comparison with the pancreatic one ($\mathbf{IC_{50}}$ >50 μM). These results suggest that the compounds may be effective in colon cancer cell lines, but to further evaluate the selectivity profile of the compound, additional cytotoxicity assays with non-malignant and malignant cancer cell lines would be required.

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DEVELOPMENT OF NOVEL SELENOCOMPOUNDS BASED ON GARLIC ALLYLIC COMPONENT, AS ANTICANCER AGENTS

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Selenium is an important trace mineral and compounds containing this chemical element have shown well-established chemopreventive and therapeutic effects. Likewise, many synthetic derivatives of natural products have been found to exhibit biological activity towards cancer such as garlic-based compounds. Taking all of the above into account we pursued to design Se-allyl hybrid compounds in order to increase efficacy as compared to naturally occurring allylic compounds. A total of 18 new selenoallyl (SeAl) compounds were designed following two different synthetic approaches. SeAl functionality was chosen to mimic the garlic bioactive allylic components. All the synthesized compounds were screened against colorectal (HT29 and HCT116) and mammary (MCF-7) cancer cell lines (48h treatment). Four most active compounds identified were submitted to the NCI Developmental Therapeutic Program (DTP). Two out of the four compounds were selected for the dose-response assay against a panel of 60 cancer cell lines. Both the compounds exhibited impressive cytotoxicity - lethal dose 50 (LD_{50}) values below 9.2 μM in most of the melanoma and ovarian cancer cell lines tested (8 out of 9 for melanoma and 5 out of 7 for ovarian cancer cells for both compounds). Additionally, the Se-allyl derivative bearing an aspirin substituent presented a significant tumor size inhibition in a castration-resistant prostate cancer xenograft model at a dose of 4 mg/kg given intraperitoneally. Thus, this compound has the potential to be a promising candidate for prostate cancer treatment and potentially other cancers. To conclude, we firmly believe, based on the evidences obtained and presented herein, that structural modifications involving the hybridization approach with Se and naturally occurring allylic compounds could stand as a novel toolkit for anticancer drug development.

BIOLOGICAL EVALUATION OF BIO-MIMETIC CHITOSAN NANOFIBROUS ELECTROSPUN NANOFIBERS

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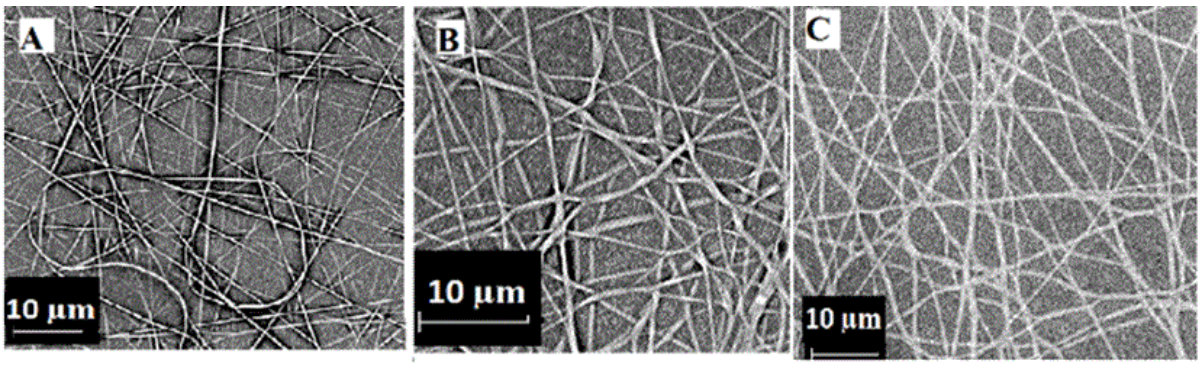
Purpose: Nanotechnology offers a superlative approach to accelerate the healing of acute and chronic wounds by stimulating the various phases of healing. In nanotechnology, nano-sized materials are used to administer topical drugs to heal wounds. The aim of this paper is to develop new electrospun nanofibers based on chitosan and polyethylene oxide (CS/PEO) and to evaluate their antiradical ability as new dressing materials in the treatment of wounds.

Materials and methods:The preparation of CS/PEO matrices was done in two stages: (i) the formation of 3 biopolymeric solutions starts by dissolving CH and PEO in 50% acetic acid by stirring at room temperature. The two solutions will be mixed in appropriate ratios, then, over the resulting mixture, the active substances (arginine and propolis) were added and stirred until a homogeneous solution is obtained; an INOVENSO nanospinner was used, a needle syringe of appropriate size filled with the polymer solution and then different values of flow-rate, applied voltage and also different distances from the tip of the syringe to the collecting plate were applied, depending on each sample. The 3 nanofibrous matrices developed were: PEO/CS/Arg (A); PEO/CS/Arg-dry Propolis (B); PEO/CS-Arg-alcoholic solution Propolis (C) as seen in the SEM micrographs below.



The evaluation of the antiradical ability was performed using the free radical DPPH and the cation radical ABTS $^{+}$. The antiradical capacity was calculated as a percentage of inhibition (I%) using the formula: I% = (A0 - As/A0) x 100 where, A0 = the absorbance value of the 0.1 mM DPPH methanolic solution / ABTS+ ethanolic solution; As = the absorbance value of the formulation, read 30 minutes or 60 min after the addition of the DPPH methanol solution / read 6 min after the addition of the ABTS^{+} solution. The evaluation of the anti-hemolytic potential was also performed by a spectrophotometric method, by the ability to stabilize erythrocyte membranes at lysis induced by hypotonicity.

Results and discussions: Following the research, new electrospun-nanofiber materials were evaluated, based on chitosan and various active substances, in terms of antioxidant activity, using 2 in vitro tests and in terms of anti-hemolytic potential. After analyzing the data obtained, it was concluded that nanofibers with propolis incorporated as a subst. solid obtained the best results.

Conclusions: The studies and results obtained justify the evaluation of the biological, antibacterial and pro-healing potential in the treatment of various wounds, starting from the antibacterial/antioxidant effects of chitosan and the beneficial role of topical propolis applied in the treatment of wounds.

Acknowledgments: The work has been funded by the Operational Programme Human Capital of the Ministry of European Funds through the Financial Agreement 51668/09.07.2019, SMIS code 124705

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MICROFLUIDIC-BASED BLOOD COLLECTION TECHNOLOGY FOR THERAPEUTIC DRUG MONITORING OF PATIENTS WITH DEPRESSIVE DISORDERS

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Depression is currently considered one of the most leading causes of disability worldwide, with people of all ages affected, which may begin with mood fluctuations and become a serious health condition. Antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), can be an effective form of treatment for moderate-severe depression. Although SSRIs were the first “new-generation" antidepressants to be introduced into the market, they are currently among the most prescribed drugs to treat depression and related disorders [1]. However SSRIs can cause some significant side effects, and for this reason, it is important to reach personalisation of therapies according to the peculiar clinical profile of patients, in the perspective of precision medicine. To this aim, an accurate and frequent therapeutic drug monitoring (TDM) is essential to evaluate therapy efficacy due to wide variability among individuals, while reducing adverse and toxic effects especially in cases of polypharmacy. A dried microsampling approach based on an innovative microfluidic channel technology was proposed within this research for TDM purposes. This strategy exploits the possibility of collecting a precise 10-µL capillary whole blood volume in a minimally invasive way by means of fingerprick and once completely filled, the microfluidic channel generates a fixed-volume dried blood spot (DBS) regardless of blood density. In fact, blood haematocrit (HCT) could otherwise generate volumetric biases, reflecting on spot size and homogeneity, sampling reproducibility, accuracy and precision of analytical data [2]. Moreover, the developed microsampling approach allows to carry out the drying step at room temperature, as well as transport and storage, ensuring stability profiles comparable to those of cryopreserved biological fluids. An original analytical workflow was designed and developed for fluoxetine, often considered one of the most important drugs belonging to the SSRI class, and its main active demethylated metabolite (norfluoxetine). The optimised methodology based on the use of microfluidics, fast pretreatment and a sensitive LC-F-MS/MS analytical method, was validated giving satisfactory results (extraction yield >80%, precision RSD <10%) and successfully applied for the analysis of microvolumes of capillary whole blood collected from patients undergoing fluoxetine treatment. In order to assess the adherence of quali-quantitative results obtained by the proposed microfluidic-based strategy, a reference plasma procedure was carried out for comparison, showing good agreement. The developed miniaturised methodology proved to be a valid alternative for the TDM of patients with depressive disorders under fluoxetine therapy, overcoming HCT issues, but maintaining all advantages of DBS processing. The microfluidic-based platform demonstrated to be suitable for a volumetric capillary blood microsampling, possibly increasing patient compliance when compared to classic haematic analysis and leading to widespread at-home and self-sampling practices.

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NOVEL SAMPLING AND ANALYSIS APPROACHES FOR THE INVESTIGATION OF TRYPTOPHAN METABOLISM IN MICE AND ITS CORRELATION WITH AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by degeneration of the upper motor neurons in the motor cortex and the lower motor neurons in the spinal cord and brain stem, resulting in progressive paresis of voluntarily innervated muscles and impairment of mobility. The aetiology of this disorder is still unknown and no pharmacological treatments are currently available to modify the disease process. Tryptophan (TRP) metabolism is involved in the regulation of immunity, neuronal function and intestinal homeostasis, while imbalances in its metabolism in disorders ranging from cancer to neurodegenerative disease have stimulated interest in the research community to deepen the study in order to clarify pathological mechanisms and to therapeutically address new targets.

The purpose of this study, in the context of a collaborative multidisciplinary research project, is aimed at designing and developing ad-hoc instrumental analytical methodologies coupled to advanced sampling and pretreatment procedures for the quali-quantitative assessment of several TRP metabolites in haematic and alternative biological samples from ALS-bearing mice and wild type mice, in order to identify potential biomarkers of the disease progression. In particular, this part of the work is focused on the miniaturisation of sampling and pretreatment procedures for biological samples. In fact, the availability of limited matrix volumes and the perspective of reducing solvents and reagents, also in the general framework of the "3Rs" principle for animal testing (replacement, reduction, refinement), make advanced sampling and pretreatment strategies particularly attractive and promising [1].

An original miniaturised sample collection procedure based on plasma volumetric absorptive microsampling [2] was coupled to an original targeted analytical method exploiting liquid chromatography and mass spectrometry (LC-MS/MS) for accurate and precise quantitative evaluations by using multiple reaction monitoring (MRM) mode. Moreover, untargeted strategies based on high resolution mass spectrometry (UHPLC-HRMS) are developed in order to add a complementary point of view and maximise the coverage of metabolic species detected and quantified in complex biological matrices. The microsampling approach and the instrumental analytical methodologies have been fully validated on a wide set of TRP metabolites, obtaining promising results in terms of sensitivity, extraction yields, precision and accuracy. The methodology is being applied for the analysis of plasmatic microsamples coming from mice bearing ALS and wild type controls and will allow the measurement of changes in metabolism, in order to evaluate the presence of specific TRP metabolites that could potentially have a role in the onset/progression of the disease and hopefully represent new promising therapeutic targets for ALS.

This research was financially supported by the Research Projects of National Relevance (PRIN) 2017 funds (Italian Ministry of Education, University and Research), within the Research project “Linking tryptophan catabolism to amyotrophic lateral sclerosis: from the pathogenesis to the pharmacological treatment” (20173EAZ2Z).

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AN ATR-FTIR METHOD FOR FAST ANALYSES OF CANNABIS PRODUCTS

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Cannabis is becoming increasingly prevalent worldwide for both medical and recreational purposes. Aside from regulated markets, the illicit one continues to be a factor. The widespread use of new products on the market, such as hemp with low concentration of Δ9-tetathydrocannabinol (Δ9-THC) and high levels of cannabidiol (CBD), or the spread of synthetic cannabinoid-based products on illicit markets, has further complicated the assessment of compliance with legal standards and enforcement issues related to *Cannabis*. For these reasons, it is essential to develop innovative methodologies to identify and discriminate different types of *Cannabis* products on the basis of their cannabinoid content. Currently, this is done through chromatographic strategies coupled to highly sensitive detection systems, such as mass spectrometry (MS, MS/MS)[1, 2]. In this study, an alternative method based on fast attenuated total reflection (ATR) Fourier-transform infrared (FTIR) spectroscopy for the identification and quantitation of cannabinoids in *Cannabis* products is proposed. ATR-FTIR allows to directly analyse even minute amounts of cannabis inflorescences without the need for any sample pretreatment and thus, in a non-destructive way. ATR-FTIR technology has been herein applied for the identification of different classes of active compounds in *Cannabis*, highlighting the discriminatory potential of some regions of cannabinoid spectra. For this reason, ATR-FTIR spectra of standard reference cannabinoids were compared with those from *Cannabis*, in order to identify spectrum regions and bands allowing their identification and useful from a quantitative point of view. *Cannabis* spectra with known content of Δ9-THC, its acidic precursor (THC-A) and CBD were collected and the cannabinoid concentrations were statistically correlated to IR bands, in order to set up correlation curves. Then, the developed strategy was applied for the quantitation of THC in several types of *Cannabis* samples and the obtained results were compared with the concentration found by reference HPLC-MS/MS analysis. The developed ATR-FTIR methodology showed a satisfactory linearity (R^2> 0.9774), sensitivity (LOQ = 1.1 μg/mg) and precision (> 85%). This alternative method is able to grant a successful application to the direct identification and quantitation of cannabinoids, in order to discriminate among different types of plants and products, based on their cannabinoid content, thus representing a useful tool for both compliance in the regulated markets and the understanding of synthetic and illegal derived cannabinoids in the illicit market.

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KITM: MEDICINAL CHEMISTRY STORY AND STRUCTURE-BASED DESIGN OBSERVATIONS

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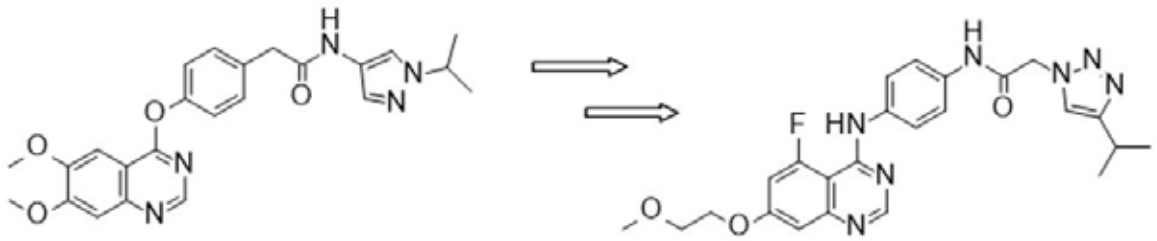
While the treatment of gastrointestinal stromal tumors (GISTs) has been significantly advanced by the application of targeted tyrosine kinase inhibitors able to inhibit KIT-driven proliferation, diverse mutations to this kinase can drive resistance to established therapies such as Imatinib. We embarked upon a drug discovery program focused on the development of KIT inhibitors which could address such resistance issues through the pursuit of a profile with strong *in vitro* inhibitory activities against multiple clinically-relevant KIT mutants.

We began our medicinal chemistry program starting from a series of previously reported phenoxyquinazoline and quinoline based 'type 2' kinase inhibitors of PDGFRα (e.g. **1**). We optimised potency against a diverse panel of mutant KIT driven Ba/F3 cell lines, with a particular focus on reducing activity against a Ba/F3 derived KDR driven cell model in order to minimise the potential for hypertension commonly seen in current second and third line GIST therapies. During the course of the medicinal chemistry development program, we investigated compounds with a structural shift to an alternative amide linker of the original series, and further establishment of SAR for this new scaffold allowed us to optimise towards more potent analogues with suitable kinase selectivity and DMPK properties.

As a result of this progress, and aided through X-ray protein crystallography showing key ligand binding modes, we identified clinical candidate *N*-(4-{[5-Fluoro-7-(2-methoxyethoxy)quinazolin-4-yl]-amino}phenyl)-2-[4-(propan-2-yl)-1H-1,2,3-triazol-1-yl]- acetamide (**AZD3229**) which demonstrates potent single digit nM growth inhibition across a range of cell lines, and a good margin to KDR-driven effects. **AZD3229** demonstrates excellent cross-species pharmacokinetics, shows robust *in vivo* pharmacodynamic inhibition of KIT mutants, and is active in several disease-relevant *in vivo* models of GIST.



1 **AZD3229**

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A COMPREHENSIVE CHARACTERISATION OF THE MOLECULAR BINDING MECHANISM OF SHELTERIN PROTEIN TPP1 TO HUMAN TELOMERASE INVESTIGATED BY COMPUTATIONAL METHODS

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Telomeres are an ensemble of proteins, noncoding DNA and RNA that protect chromosome ends from degradation and end fusions, thus being crucial for the cell life span. As part of this complex, the Telomerase enzyme (TERT) adds polynucleotide sequences at the end of telomeric DNA, preserving its length. In its action, TERT is assisted by several regulatory proteins, above all the Shelterin complex protein TPP1 [1]. Recently, the TPP1's binding region to TERT has been investigated by mutagenesis experiments and named TEL-patch. Conversely, it is still unclear which part of the TERT is implicated in this protein-protein interaction (PPI), although several studies suggest a pivotal role of the TERT's TEN domain [2]. Here, we present a thorough computational study based on homology modeling, docking calculations and molecular dynamics (MD) simulations aimed at finding reliable binding modes between TPP1 and TERT. We performed the MD calculations both on TPP1 WT and its dyskeratosis congenita related mutant (LYS170Δ) [3]. Our results show that a single deletion in TPP1 induces a severe impairment of the TPP1-hTEN PPI. Furthermore, we report preliminary results of a drug design campaign aimed at identifying the first TEL-patch ligands able to inhibit the binding with TERT. Firstly, we chose the best hits screening million of compounds, filtering them based on the molecular descriptors of PPI inhibitors and marketed drugs able to enter the cell nucleus [4]. Then, the selected hits underwent docking calculations in the TEL-patch and the binding mechanism of the best candidates was investigated by funnel-metadynamics, a powerful binding free-energy method developed in our lab [5][6]. The resulting compounds are under ongoing *in-vitro* test evaluation. If successful, our study will provide the first inhibitors of the TPP1/TERT PPI, paving the way to the development of a new generation of anticancer drugs.

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COVALENT INHIBITION OF BACTERIAL UREASE BY BIFUNCTIONAL CATECHOL-BASED PHOSPHONATES AND PHOSPHINATES

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Ureolytic activity of various pathogenic strains of microorganisms is a virulence factor for the colonization of host organism. Urease inhibitors may be used as potential therapeutic agents supporting antibiotic treatment of infections caused by microorganisms which express urease. A novel group of bifunctional urease inhibitors was synthesized for this study. Two functional residues – phosphonate or (2-carboxyethyl)phosphinate as well as catechol group – were both located on a short propionate scaffold. β-substituting phosphorus-based organic moieties were meant to engage in coordination complexes with catalytic nickel cations in urease while the catechol residue was earlier confirmed to bind covalently to the thiol group of Cys322 in the enzyme's active site. The inhibiting activity against native urease purified from bacterial culture as well as ureolysis in whole cells of *Proteus mirabilis* was measured. The compounds exhibited varying structure-dependent activity and kinetic mechanisms. The structure of methyl β-(3,4-dihydroxyphenyl)-β-(2-carboxyethyl)phosphorylpropionate was the most effective irreversible urease inhibitor (k_{inact}/K_I = 12700 $s^{-1}M^{-1}$) in the studied group. Modeling studies confirmed that this particular molecule exhibits a perfect fit into the active site of *Sporosarcina pasteurii* urease and thus shows the potential for double-action binding mode.

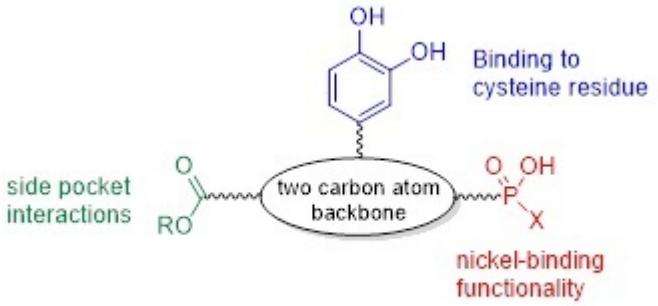

Figure 1. A general structural interaction mechanism of potential bifunctional urease inhibitors

The work was supported by National Science Centre grant 2018/31/B/NZ6/02017

DESIGN IMPROVED NOVEL LIGANDS BY COMBINING LIGAND AND STRUCTURE-BASED APPROACHES

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After identifying novel bioisosteres of a known inhibitor of Tankyrase, using ligand-based shape and Electrostatic Complementarity™ the results are explored and ranked using protein-ligand Electrostatic Complementarity together with docking scores. Coupling these methods exploits the rapid screening power of the ligand-based method then facilitating compounds to be optimized interactively in the active site.

OPTIMIZED SEMI-INDUSTRIAL ELECTROCHEMICAL PREPARATION OF CYCLIC ENCARBAMATES AND ITS APPLICATION FOR SYNTHESIS OF MEDCHEM RELEVANT BUILDING BLOCKS

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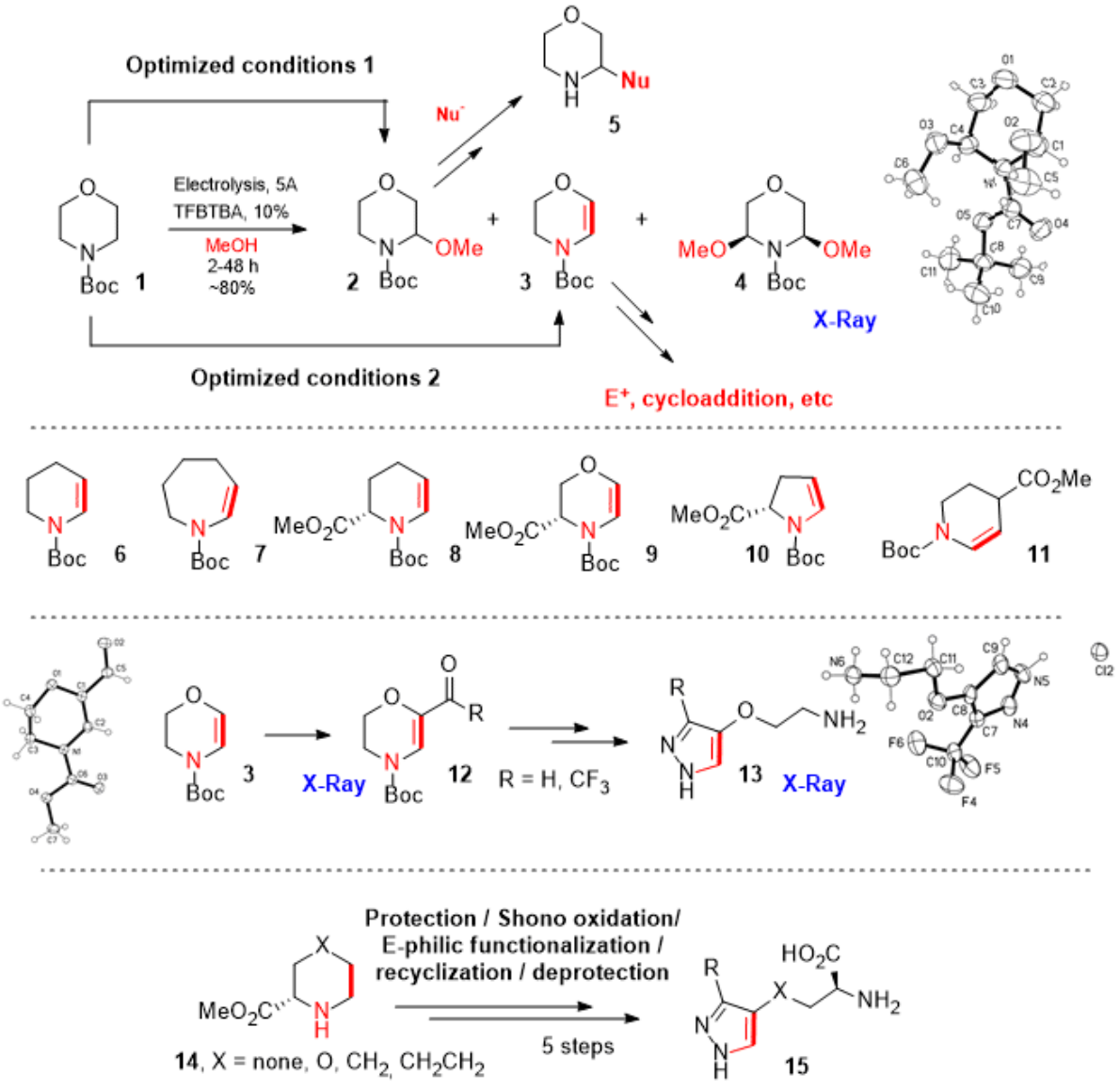
The well-known electrochemical Shono oxidation of Boc-protected cyclic amines was revised. Boc-protected morpholine **1**, which in described conditions gives the mixture of products, was considered as model compound. The process was optimized for synthesis of carbamate **3** in 500g from one synthetic run. The optimized procedure was applied to multigram synthesis of carbamates **6-11**. Further electrophilic functionalization of the carbamates leads to latent 1,3-bielectrophilic compounds type **12**, which were subjected to classical heterocyclization with binucleophilic reagents. In a case of hydrazine the corresponding pyrazoles **13**, decorated in unusual manner were prepared. The proposed methodology appears to be a straightforward tool for design and synthesis of MedChem relevant building blocks. For example, the five-step synthesis starting from commercially available cyclic α-aminoacids type **14** leads to α-aminoacids **15**with various linkers between aminoacid fragment and pyrazole nucleus. The scope and limitation of above mentioned approach will be discussed.



STUDY ON THE SELECTIVE SYNTHESIS OF HIGHLY SUBSTITUTED BENZOIC ACID, BENZALDEHYDES AND BENZENEBORONIC ACIDS FROM ASYMMTERIC BENZENEDIBROMIDES ON MULTIGRAM LABORATORY SCALE

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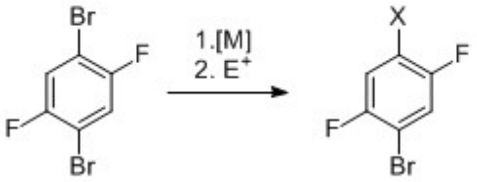
We recently developed a facile protocol to synthesize bromodifluorophenols, -iodobenzenes and -boronic acids (**1a-c**) from symmetric dibromodifluorobenzenes (e.g **2**) in good to very good yields (Scheme 1).

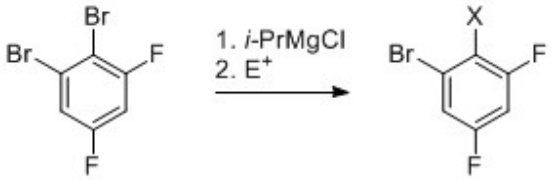
In our ongoing efforts to make a range of versatile and unique new building blocks for we have now extended this protocol to asymmetric dibromobenzenes **3**-**7** (Figure 1).



2 **1a-c**

1a X= OH ; **1b** X= I; **1c** $B(OH)_2$

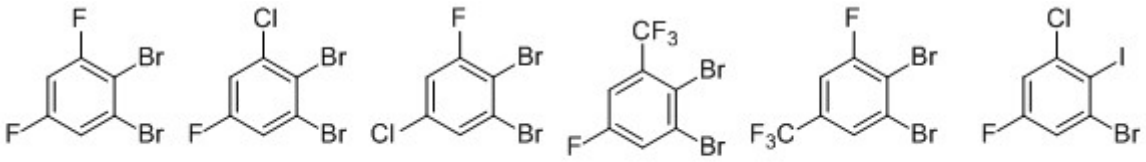
Scheme 1. study



3 **9-10**

X= CO_2H **9**; X= $B(OH)_2$ **10**

Scheme 2. This study



3 **4** **5** **6** **7** **8**

Figure 1. Asymmetric dibromobenzenes used in the study

The poster will show the synthesis of **3**-**7** and detail our findings on the selectivity of the metal/halogen exchange to synthesise a variety of building blocks (Scheme 2).

Challenges, solutions, yields and limitations will be discussed. Selectivity and yields will be compared to the corresponding iodobenzenes (e.g. **8**) where appropriate.

To the best of our knowledge only one of the products (**9**) has been reported in literature before and was made utilizing a more complex synthetic route.[2]

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DESIGN, SYNTHESIS AND MOLECULAR MODELING OF NOVEL IMIDAZO[4,5-c]PYRAZOLES DERIVATIVES AS POTENT α-AMYLASE INHIBITORS

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Despite imidazole and pyrazole signify key motifs in diverse pharmaceutically active compounds, the imidiazopyrazole scaffold is scarcely investigated for the inhibitory activity of α-amylase, a key enzyme that plays a significant role in the digestion of dietary starch into simple monosaccharides. For this purpose, we designed to unite two effective cores: imidazole and pyrazole as a backbone structure, which was tested *in vitro* for the inhibition of the α- amylase enzyme and recognized as a lead inhibitor (IC_{50} 21.2 μg/mL) with a 1.6-fold inhibition potency over Acarbose (IC_{50} 34.71 μg/mL). Based on this result, a novel series of annelated imidazopyrazoles has been designed and synthesized through various strategies. Such new scaffolds were verified by spectral data and screened for *in vitro* their α-amylase inhibition potentials. Most analogs revealed excellent to good results, which were nearly comparable and even better than standard medication Acarbose. Of these analogs, compounds **1, 5, 7, 11**, and **12** exhibiting IC_{50} values ranging from 21.2-30.49 μg/mL were discovered to be more potent than the standard drug Acarbose (IC_{50} 34.71 μg/mL). The molecular docking of the most potent compounds (**11** and **12**) against α-amylase was performed to investigate their mode of binding. The results of docking studies revealed that the docked compounds have good binding affinities against α-amylase with binding free energies of -4.30 and -4.94 kcal/mol, respectively. These energy values were near to Acarbose, (reference drug), which showed binding energy of -8.69 kcal/mol.

SYNTHESIS OF NEW BUILDING BLOCKS ON 100 G TO 1 KG SCALE. CHALLENGES AND DIFFERENCES TO SMALL SCALE REACTIONS

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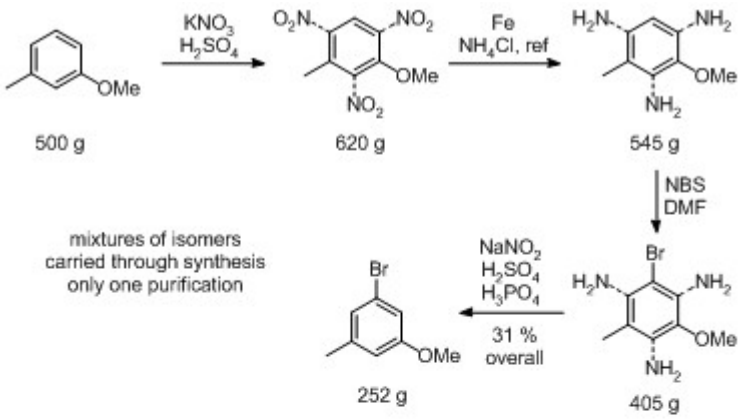
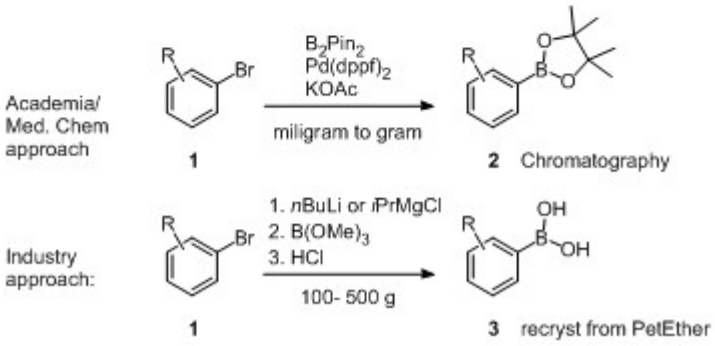
As chemists we all are familiar with small scale reactions ranging from milligrams to a few grams, for example in academia or in hit to lead optimizations in medicinal chemistry projects. Large scale process chemistry projects, e.g. the synthesis of API's on industrial scale, are documented in the primary patent literature. Interestingly, looking at the scale in between, reactions on 10's of grams to 1 kg are rarely reported in the literature or presented at conferences and symposia.

The poster will focus on the challenges and differences the synthesis of new building blocks for medicinal chemistry projects and the pharmaceutical industries on this scale contains, including the choice of reagents, difficulties of reaction analysis and a discussion on purification vs. turnover/yield and the influence available purification techniques have on the route design to new products. We will also take a close look at reproducibility of results.

The poster will feature several recent projects to make novel building blocks (e.g. anilines, boronic acids) and will include large scale lithiations and the "halogen dance", synthesis of 1,3,5-substituted electronic rich benzenes, synthesis of orthogonal substituted benzenes bearing all 4 major halogens, and a few examples on flow chemistry.



Scheme 1. 1,3,5 substitution of activated arenes



Scheme 2. Boronic esters vs Acids, advantages and difficulties for both routes

SYNTHESIS OF CYSTOBACTAMID ANALOGS AS ANTIBIOTICS

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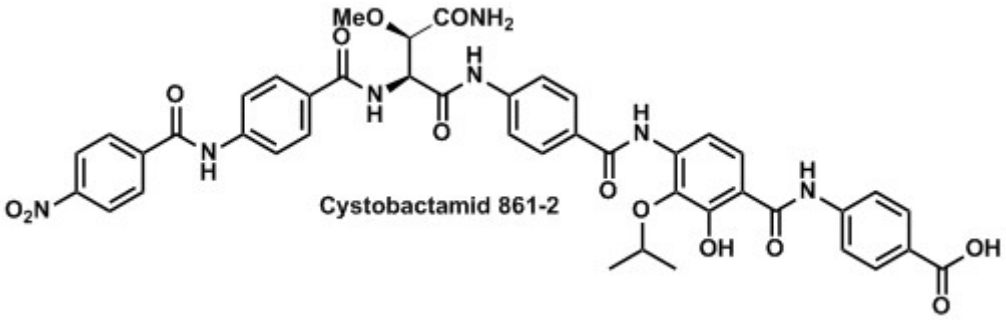
Because there is a concerning rise of multidrug-resistant human pathogens, there is a strong need for novel chemical scaffolds with resistance-breaking properties. Cystobactamids, natural products isolated from myxobacterial *Cystobacter sp.*, are a new class of antibiotics and inhibit gyrase and topoisomerase IV as key enzymes in the replication of bacterial DNA [1],[2]. Through chemical synthesis, a structure-activity-relationship has been established[3] and compounds with improved broad-spectrum activity, potency and resistance breaking properties *in vitro* compared to the natural product 861-2 were found. For example, the minimal inhibitory concentrations (MIC) against *A. baumannii* and *S. aureus* were decreased to 0.02 µg mL^{-1}, which reflect an increase in activity by 50-fold and 12-fold, respectively. On the poster, we will present strategies for analog synthesis together with a profound characterization of microbiological properties. The results qualify cystobactamids as a promising new class of antibiotics for the treatment of critical pathogens.



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BIOACTIVE COMPOUNDS, ANTIOXIDANT AND ANTIMICROBIAL POTENT OF CORCHORUS OLITORIUS . L

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Corchorus olitorius Linn (Tiliaceae) is an important edible plant in many Arab countries such as Egypt or Sudan [1], and extreme east of Algeria, it is used for the preparation of a very popular hot soup (called molokhia). In West Algeria, this plant is reported to be used, for the first time, for medicinal purposes. [2]

The evaluation of phytopharmaceutical, antioxydant and antimicrobial activities still a useful and interesting task, especially for unknown and less used medicinal plants in traditional herbal medicine. These plants represent a new source of active compounds. [3]

In this work, polyphenolic potential and *in vitro* biological activities of *Corchorus olitorius* .L will be investigated and some preliminary results will be given.

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ANTI-ALLERGIC ACTIVITY OF VARIOUS NATURAL PRODUCT SAMPLES FROM UKRAINE

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Highlights. Several plants from Ukraine showed promising anti-allergic and anti-inflammatory activities

ALLERGY: dramatically increasing incidence of allergic diseases; affecting approximately 20% of the world population; serious burden to the quality of life.

ALLERGIC REACTION: mast cells are crucial for execution of allergic reaction; activation by an antigen (usually a common environmental entity); degranulation => histamine, β-hexosaminidase and other mediators are released triggering allergic symptoms.

DEGRANULATION ASSAY: release of β-hexosaminidase from mast cells (RBL-2H3) activated by calcium ionophore A23187 or antigen (IgE plus DNP-BSA); useful tool in evaluating anti-allergic effects [1].

NATURAL PRODUCTS: untapped potential of natural products as an inexhaustible mine of active compounds, usually with a good safety profile; impuls to investigate anti-allergic activity and anti-inflammatory activity.

HERBS FROM UKRAINE: plants from *Iridaceae* family (*Crocus* sp., *Gladiolus* sp., *Iris* sp.) have been traditionally for the treatment of infections; they accumulate many secondary metabolites including flavonoids, isoflavonoids, chalcones, and xanthone; *Crocus* stigmas contain also apocarotenoids; herbs from Ukraine such as

Iris hungarica, *Iris variegata*, *Gladiolus hydrida* and *Juno bucharica* [2,3] were scarcely studied.

Results & Discussion. Previously, we found antivirus potential of different herbal extracts of *Iridaceae*, volatile oils and pure compounds.

In this study, 15 plant extracts of *Iridaceae* family obtained from Ukraine were evaluated for anti-allergic and anti-inflammatory activities. The results showed that extracts of saffron corms markedly inhibited degranulation of mast cells stimulated by A23187 or antigen, with IC_{50}range 38-79 μg/mL. The profound inhibition of degranulation induced by calcium ionophore A23187, which bypasses high-affinity IgE receptor FcεRI interaction, suggested the effect of saffron corms on calcium pathway.

Saffron flowers and leaves inhibited superoxide anion generation in human neutrophils.

The herbal natural products exerted promising anti-allergic activity and further evaluation is needed to understand the active ingredients responsible for the effects.

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DETERMINING THE SUBSTRATE SPECIFITY AND DETERMINIG THE CONDITIONS FOR THE ACTIVITY OF THE HTRAHP PROTEASE

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The bacterium *Helicobacter Pylor*i has been classified by the World Health Organization as a class I carcinogenic agent. This pathogen has infected almost half of the population in the world, but the vast majority of these people do not develop the disease. Infection with this bacterium carries the risk of diseases such as type B gastritis, peptic ulcer disease, gastric cancer or gastric mucosa lymphoma [1,4]. The cutting process is responsible for the development of gastric cancer E-cadherin, one of the key proteins responsible for connections between epithelial cells. It is digested by the protease secreted by *H. pylori* bacteria (High-temperature requirement A), which loosens the structure of the tissue, thus enabling its penetration by the bacteria. [2,3]. The aim of my project is to find a substrate for the HtrA enzyme as well as activators of this enzyme, and then the synthesis of the inhibitor. To do this, I initially synthesize a peptide library in which peptides serve as small-molecule substrates during digestion by the HtrAHP protease, in order to determine the enzyme cleavage site and determine the conditions of its activity. Studies of the HtrA enzyme carried out on the rCdh1 cadherin model showed that this enzyme is specific for the fragments with the sequence [VITA] - [VITA] -x-x-D- [DN], whereby hydrolysis occurs between two hydrophobic amino acid residues found in The *N*-terminal part of these sequences [3,5].

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DESIGN, SYNTHESIS, AND PHARMACOLOGICAL VALUATION OF TETRAC NANOPARTICLES, ANGIOGENESIS MEDIATORS

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The laboratory completed the bench-to-bedside preclinical development of Tetraiodothyroacetic acid (tetrac) and tetrac nanoparticles (tetrac NP). Tetrac blocks angiogenic and tumor cell proliferation actions of thyroid hormone initiated at the cell surface hormone receptor on integrin alphavbeta3. **(Figure 1)** Tetrac also inhibits angiogenesis initiated by vascular endothelial growth factor and basic fibroblast growth factor. Esterified and activated Tertrac were demonstrated to be chemically conjugated to a terminal end group of modified poly(D,L-lactic-co-glycolic acid) [PLGA].[1] In order to do so PLGA was first formulated into microspheres by a single in-water emulsion technique. The loading efficiency was almost 100% due to the covalent linkage of the target drug to PLGA. Because the drug release rate from microspheres was expected to be dependent on the chemical degradation rate of PLGA chain, the chemical conjugation approach was expected to be more suitable in order to get high drug payload and efficient passive targetting to solid tumors. Acting via a cell surface receptor, tetrac and tetrac NP inhibited growth of h-MTC cells and associated angiogenesis in the chick chorioallantoic membrane (CAM) assay **(Figure 2)**.

One of the challenges towards access to the clinical assessment of these biomacromolecules resided in the selection of appropriate carriers and to protect, activate, deliver and release them *in vivo* to maximize their pharmacological effects.[2]

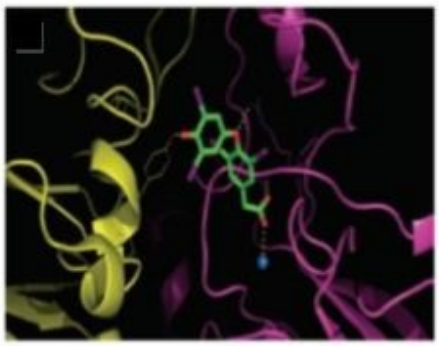

Figure 1 : Tetrac docked on integrin alphavbeta3

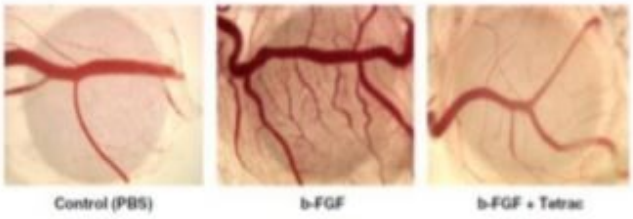

Figure 2: results of the CAM assay

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